A study to analyze reproductive potency of butter catfish, *Ompok bimaculatus* (Bloch 1794) and its induced breeding

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE IN FULFILLMENT OF

Doctor of Philosophy

IN

APPLIED ANIMAL SCIENCES

Under the Supervision of

Dr. ABHA MISHRA
Assistant Professor

Submitted By

ANURAG RAWAT

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

DEPARTMENT OF APPLIED ANIMAL SCIENCES
SCHOOL FOR BIOSCIENCES AND BIOTECHNOLOGY
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025 (U.P.), INDIA

Enrollment No. 137/08 **Year 2017**

Dedication

I dedicate this thesis to my empyreal parents and my family who have always been there to support, encouragement and prays.

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

बाबासाहेब भीमराव अम्बेडकर विश्वविद्यालय
(केन्द्रीय विश्वविद्यालय)
विद्या विहार, रायबरेली रोड, लखनऊ - 226025
Babasaheb Bhimrao Ambedkar University
(A Central University)
Vidya Vihar, Raebareli Road, Lucknow-226025

CERTIFICATE

This is to certify that the thesis titled **"A study to analyze reproductive potency of butter catfish, *Ompok bimaculatus* (Bloch 1794) and its induced breeding"** submitted by Mr. Anurag Rawat is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations - 1999 as amended in 2010* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: Supervisor

Head of the Department

DECLARATION

I hereby declare that this thesis entitled **"A study to analyze reproductive potency of butter catfish, *Ompok bimaculatus* (Bloch 1794) and its induced breeding"** submitted by me under the supervision of Dr. Abha Mishra, in accomplishment of the degree of Ph.D. in Applied Animal Sciences, Department of Applied Animal Sciences, School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow (U.P.). It is an outcome of my own efforts and is an original research work.

Date: *(Anurag Rawat)*

Place: Lucknow

Acknowledgement

I would like to offer my heartfelt salutation to almighty God for the unbroken health and vigor, bestowed me, all through the span of my studies in accomplishing this arduous work. I am immensely rejoiced to owe my deep sense of gratitude to Hon'ble Vice Chancellor, Prof. R. C. Shobti, Babasaheb Bhimrao Ambedkar University, Lucknow, to providing me an opportunity to work in an esteemed institution.

I feel great pleasure to evince my profound sense of veneration and gratitude to Prof. S. Sharaf (Dean), Dr. V. Elangovon, (Head), Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, for their support and providing laboratory facilities throughout my research study.

I have paucity of words expressing my gratefulness to my supervisor Dr. Abha Mishra, Assistant Professor, Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, for her guidance and constant supervision during the course of study.

It's my genuine abiding desire to express my earnest gratitude in acknowledging Prof. K. Jaiswal , Dr. Venkatesh Kumar R., Dr. S. Mishra, Faculty of Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, for their warm blessing for commencements in course study and support where ever it was required.

I am grateful to Dr. U. K. Sarkar, Head of Division and Principal Scientist, Central Inland Fisheries Research Institute, Kolkata for his kind co-opration and stimulating discussions, valuable suggestions and other indefatigable efforts.

I would fail in my duty for not acknowledging the cooperation of couples included in my study. My special thanks to my beloved wife and colleague Mrs. Sneha Verma for their generous attitude and kind cooperation rendered to me during the course of my thesis.

It is an immense pleasure for me to express my sincere acknowledgement to Agromet Observatory, Indian Institute of Sugarcane Research, ICAR, Lucknow to provide meteorological data for my research.

I would like to thanks the non- teaching staff of Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, for their cooperation during the course of my study.

I would also like to express my gratitude to some skilled fishermen, Mr. Birju, Mr. Ramu, Mr. Ramsanehi, Mr. Mukesh and Mr. Radhe Shyam, for providing me healthy experimental fish.

Many more people helped me during span of times. It will be not fair if I do not list them all. It is not lack of gratitude but for lack of space that I keep my pen down to them all I convey my deep love and affection.

Anurag Rawat

Table of Contents

S. No.	Contents	Page No.
1.	**General Introduction**	1-10
2.	**Chapter 1** Exploration of morpho-metric and reproductive parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in different Indian rivers	11-64
3.	**Chapter 2** Annual reproductive cycle of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in Lucknow region	65-111
4.	**Chapter 3** Induced breeding performance of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) with different hormones	112-133
5.	**Consolidated Summary and conclusion**	134-135
6.	**References**	136-177
7.	**List of Publications**	———

General Introduction

Fish are an economic and easily digestible source of protein over one billion people. Fish considered as a fifth largest agricultural resource. It constitutes the significant part of thirty-eight thousand vertebrates perceived all over the world. Nelson (2006) estimated that 27,977 species of fish are valid worldwide which came under 62 orders, 515 families and 4,494 genera, and the number of extant fish species was close to 32,500. About 11,952 species are usually live in freshwater lakes and rivers. In our Indian region, there are two thousand and five hundred species, of which nine hundred and thirty are inhabitants of fresh water and the rest in seas (Jayaram, 1999).

Fish are the keystone species used as an indicator of good water quality and health of the aquatic environment (Bijukumar, 2000). They control the distribution as well as the richness of other organisms in the aquatic ecosystems. India is sanctified with different natural water resources in the form of rivers, streams, reservoirs, wetlands, lakes and ponds, etc. that all are natural habitats for fish species.

1. Indian Riverine System: A study area

Ayappan and Birdar (2004) stated that there are various freshwater fishery sites like rivers (45,000 km), canals (1,26,334 km.), ponds, tanks (2.36 million hectares) and reservoirs (2.05 million hectares).

In Indian riverine system, the most important river is **Ganga River** which is the fifth largest river in the world (Welcomme, 1985). The Ganga flows through the India and Bangladesh. The River originates from ‘Gaumukh' in the Garhwal Himalaya and ends up into the Bay of Bengal. Extreme variation in flow exists within the catchment area, and the river transported a vast amount of sediment and distributed across the fringing floodplains during the monsoon. The basin of river Ganga has very high cultural, heritage and religious values. It supports rich biodiversity, though out of 30 river interlinks have been identified (Lakra et al., 2010a, b). The Ganga River Basin is located

70-88°30' east and 22°-31° north. The source of water in the Ganga are direct seasonal rainfall, mainly from the south-west and glacial and snowmelt during the summer. The main River Ganga is the combination of two rivers, the Bhagirathi originating at Gaumukh from Gangotri glacier and the Alaknanda originating at Satopanth glacier in Garhwal Himalayas. The two rivers flow separately for about 200 km and receive several small tributaries before they merge at Dev Prayag where it attains the name of Ganges. In the plains, the Ganges receives many tributaries such as Ramganga, Gomati, Ghaghara, Gandak and Kosi on its left bank. The Mahananda, another major tributary, joins the Ganges in Bangladesh. Besides, many tributaries such as Yamuna, Tons, Sone, Punpun, Kiul and Ajay accede to the Ganges at its right bank. River Damodar, Rupnarayan and Haldi join the Ganges downstream Kolkata. The total length of the Ganges from Gaumukh to its mouth at Sagar Island in the Bay of Bengal is 2715 km (Singh, 1996; Parua, 2001).

River Brahmaputra is one of the major rivers of Asia. It originates in the Kailas range of the Himalayas at an elevation of 5150 m in China under the name of Yalung Zangbo. After flowing for 1700 km parallel to the main range of the Himalayas, it enters India, and after passing through 720 km in Assam, it enters Bangladesh below Dhubri and after traversing for 279 km joins the Ganges (Padma) at Goalundo in Bangladesh (Rao, 1979). It flows Southwest through Assam Valley as Brahmaputra and South through Bangladesh as the Jamuna. This river is about 2900 km long. The Brahmaputra River system is a virtual lifeline to the seven North-Eastern States of India where it covers an area of 5,80,000 km^2.

The **River Cauvery** is one of the major river of India. It originates at Talakaveri in the Western Ghats in the state of Karnataka, to Tamil Nadu and across the Southern Deccan plateau, emptying into the Bay of Bengal. Cauvery River has many tributaries including Shimsha, Hemavathi, Arakavathy, Honnuhole, Lakshmana, Tirtha, Kabini, Bhavani, Lokapavani, Noyyal, and the Amaravathi (Jayaram, 1982; Begum and Harikrishna, 2008).

River Krishna is the major river of South-central India. It originates from Maharashtra, flows through the Maharashtra, Karnataka, Telangana and Andhra Pradesh and meets the sea in the Bay of Bengal. The length of river Krishna is nearly 1,400 km east-coast. Vijayawada is the largest city and situated on the bank of river Krishna (Jain et al., 2007).

The **Mahanadi River** is the third largest in the peninsular region of India. The main tributaries are the Suktel, the Jeera, the Jonk, the Ibb, the Ong, and the Tel (Hora, 1940).

Narmada River, a west flowing river, is the fifth largest (Vyas and Vishwakarma, 2013). River Narmada originates from Amarkantak (Madhya Pradesh) and meets in the Gulf of Cambay in Arabian Sea (Gujarat). River Narmada supports a wide variety of living organisms that inhabit different stretches of the river depending on the ecological conditions (Vyas and Vishwakarma, 2013).

River Subernrekha originates in the Chotanagpur plateau near Ranchi and meanders through the states of Jharkhand, West Bengal and Orissa before draining into the Bay of Bengal near Kirtaniya/ Chaumukha in the district of Baleswar (Balasore) in Orissa (Karmakar et al., 2008).

Among the tributaries of Indian riverine system, **Betwa River**, the river of Northern India is a tributary of the Yamuna River. The Betwa falls in the Bundelkhand region in central India. It originates in the Raisen district of Madhya Pradesh and joins River Yamuna near Hamirpur in Uttar Pradesh, travelling a total distance of about 590 km (Lakra et al., 2010a, b). The **Chambal River** is a tributary of the Yamuna River in Northern India (Yadav et al., 2014). The Ghaghara River is a major tributary of the Ganga river system in Northern India. In Uttar Pradesh, Ghaghara flows in a South-East direction to the town of Chhapra where after a course of 570 miles (917 Km) it joins the Ganges. The major tributaries of **Ghaghara** are Rapti, Chhoti Gandak, Sharda and Sarju (Sarkar et al., 2012). The river **Ramganga, Sharda** and **Gomati** (a major tributary of the Ganga river basin in Northern India) is the tributary of River Ganga, and it supports

livelihood for freshwater fish and nutritional security (Sarkar et al., 2012). The River Ramganga is one of the principal rivers from the Shiwaliks. Khoh, Kolhu and Mandal are tributaries of the Ramganga. Sharda River rises in Lakhimpur Kheeri in the Terai-belt of Uttar Pradesh-India (Atkore et al., 2011). **Sone River** is one of the largest of the Ganges Southern tributaries. **Hooghly River** is a tributary of the Ganges River in West Bengal, India. It splits from the Ganges as a canal in Murshidabad District at the Farakka Barrage. **River Tapti** is one of the major rivers flowing in the western part of India. It originates in Satpura Mountain in Betul district of Madhya Pradesh and runs through Madhya Pradesh, Maharashtra and Gujarat before it meets the Arabian Sea (Lakra et al., 2010a, b).

Several researchers have studied the fish and biodiversity of the river Ganges and its tributaries (Hora, 1929; Srivastava, 1980; Bilgrami and Munshi, 1985; Krishnamurti et al., 1991; Revenga and Mock, 2000; Payne et al., 2004; Sinha, 2006; Sarkar et al., 2010). The freshwater ecosystem is facing serious threats. Recent estimation of Maclean and Jones (1995) and IUCN (2010) suggested that 20 percent of all freshwater species are extinct, endangered or vulnerable. There is a loss of aquatic ecosystems and therefore fish biodiversity (Ricciardi and Rasmussen, 1999; Gibbs, 2000; Dawson et al., 2003).

Freshwater fish is one of the threatened taxonomic groups (Darwall and Vie, 2005; Dudgeon et al., 2006; Sarkar et al., 2008). The disturbance in the ecosystem is especially due to human interference (Abell, 2002). Another important reason for their extinction is the limitations in the physiology, morphology, and life history of species associated with environmental constraints (Williams et al., 2003; Hilbert et al., 2004; Skov and Svenning, 2004; Thomas et al., 2004). Because of the sensitive nature of fish and habitat disturbance, they often used as bioindicators for the water quality (Chovance et al., 2003).

2. Experimental fish: Ompok bimaculatus (Bloch, 1794) (Near Threatened fish)

The catfish are an important part of the fish fauna, and many of them are economically important with high nutritive value (Rajagopal and Davidar, 2008). The

diversity of catfish in India is highest in North Eastern hills, Gangetic River system and the Western Ghats (Barman, 1994; Thomas et al., 2002).

The freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) locally known as pabda, is an indigenous species. It is popularly known as butter catfish because of its flexible bony structure. It has extensive geographical distribution covering South East-Asia (India, Pakistan, Afghanistan, Myanmar, Thailand, Java, Sumatra, Borneo and China (Talwar and Jhingran, 1991; Jayaram, 1999). It has real demand as food material because of its good taste and rich lipoprotein content and single line skeletal structure (Banik et al., 2011). They are usually carnivorous and insectivorous in nature but occasionally feed on crustaceans and planktons (Parameswaran et al., 1970). This fish species is widely distributed along the plains and sub-mountain regions in natural water bodies, i.e. horse (large natural depressions), bars (oxbow lakes), rivers, bees (low-lying seasonal water bodies) and floodplains (Rahman, 1989; Riehl and Baensch, 1991). Due to rich lipoprotein content, this fish is highly nutritious to the people.

Over the last few decades, the ecology of the riverine system has suffered from anthropogenic factors. This leads to industrial pollution, pesticide, extensive habitat destruction and defragmentation, riverine siltation, disease, pollution, poisoning, destructive fishing in breeding season, proliferation of exotic fish species and high exploitation (Arthington and Welcomme, 1995; Ricciardi and Rasmussen, 1999; Gibbs, 2000; Dawson et al., 2003; Arthington et al., 2004; Szollosi-Nagy, 2004; Dudgeon et al., 2006; Lima-Junior et al., 2006; De Silva and Abery, 2007; Chakrabarti et al., 2009; Sarma et al., 2012; Banik and Malla, 2014). According to Leveque et al. (2005) and Mas-Marti et al. (2010), the global climate change was also one of the reason for population reduction. As a consequence, the population of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) is drastically reduced and has listed as a near threatened species as per IUCN (International Union for the Conservation of Nature and Natural Resources, List of Threatened Species (Version 3.1) (Ng et al., 2010).

Therefore, to conserve and sustainable management of this near threatened catfish, induced breeding is urgently needed. So far breeding biology is concerned, and also success has been achieved in many catfish induced breeding at the captive condition in India. There are studies related to captivity breeding of catfish (Raj, 1962; Akhteruzzaman et al., 1993; Cheah and Yeo, 1994; Goswami and Sarma, 1997; Bhowmik et al., 2000; Mukherjee and Das, 2001; Das, 2002; Haniffa and Sridhar, 2002; Das and Kalita, 2003; Mollah, 2003; Mahapatra, 2004; Chakrabarty and Chakrabarty, 2005; Sahoo et al., 2005; Sarkar et al., 2005; Hossain et al., 2006; Hussain, 2006; Mahmood, 2006; Chakrabarty et al., 2006, 2007, 2008; Roy et al., 2007; Rahman et al., 2008). The Indian freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) was artificially spawned to propagate its existing population (Choudhury, 1962; Sridharan et al., 1998).

2.1. Systematic position

Group: Pisces
Phylum: Chordata
Subphylum: Vertebrata
Superclass: Gnathostomata
Class: Osteichthyes
Subclass: Actinopterygii
Division: Teleostei
Subdivision: Euteleostei
Superorder: Ostariophysi
Order: Siluriformes
Family: Siluridae
Genus: *Ompok* (Lacepede, 1803)
Species: *bimaculatus*
(Bloch, 1794)

2.2. Morphology

The catfish *Ompok*, is Silurid fish, has a short dorsal fin with four rays. Anal fin is long, inserted well behind the dorsal fin. The caudal fin strongly forked. The eye is subcutaneous, immediately posterior to the mouth rictus and two patches of palatal teeth. The body is elongated and compressed. Depressed head and the rounded snout are present. Eyes are moderate in size, its lower edge below the level of the cleft of mouth. Large and oblique mouth is present. The teeth are in villiform bands on jaws. The lower jaw is longer than upper jaw; the width of the gape of the mouth equals half the length of the head. Barbels are two pairs. The maxillary pair barbel short and extend slightly beyond to anal fin base. The mandibular barbels are very short. Nostrils are widely separate from each other. Pectoral spine is moderately strong, usually feebly serrated on its inner edge. The body color is silvery grey above and lighter below, with a dark black shoulder spot (Rahman, 1989; Talwar and Jhingran, 1991; Rahman, 2005; Parween, 2007).

2.3. Reproductive Biology

Reproduction is a very complex process that involves gametogenesis, development of reproductive organs, migration to breeding grounds, courtship behavior, breeding, etc. The study of fish reproductive biology is utmost important in pisciculture management (Mollet et al., 2000; Chakraborty et al., 2010). This help in increasing the technological efficiencies of aqua-culturist. Aquaculture management requires a complete knowledge of reproductive biology such as size at maturity, gonadal maturation, fecundity, gonadosomatic index (de Carvalho et al., 2009; Fontoura et al., 2009). Information about the reproductive season enables us to know about spawning duration (Sato and Yoseda, 2008).

Fish growth, length weight parameters, fecundity have positive influence on reproduction. In fish biology, morphometry is an important tool in general and stock identification in particular (Cadrin and Friedland, 1999; Hussain et al., 2012; Mir et al.,

2013). The complete set of measurements used to describe a form is a morphometric character set (Strauss and Bond, 1990). The knowledge of length-weight relationship can be used to obtain information for a particular fish stock assessment and aquaculture management (Morato et al., 2001; Stergiou and Moutopoulos, 2001; Gonzalez et al., 2004).

The method of studying the spawning season or reproductive biology is to follow the seasonal changes in gonadal weight in relation to body weight, expressed as the gonadosomatic index (Dadzie et al., 2000; Ahirrao, 2002). The gonadosomatic index (GSI) is a good indicator of reproductive activity, being used to determine the stages of gonadal maturation (Hojo et al., 2004). Environmental factors also influence GSI. It is helpful in fish breeding too (Belsare, 1962; Lehri, 1968; Shashi and Akela, 1996). The reproductive strategies and fecundity are important in aquaculture management and conservation (Maurice and Burton, 1984; Manna and Raut, 1991; Hunter et al., 1992; Alam and Das, 1996; Sato et al., 2003). The fecundity varies among different species and also within the same species depending upon the internal and external environment (Somvanshi, 1985; Kulshrestha et al., 1990; Barmanh and Saikia, 1995; Shinde et al., 2002). Fecundity is the actual reproductive rate of an organism or population, measured by the number of gametes (eggs) (Eugene, 1958; Etienne, 1982).

The fish have different reproductive cycle depending on the natural environmental conditions. The spawning time in annual spawner has developed as a response to maximize the eggs and fry survival of that particular fish. It depends on several ultimate factors; some of these factors include water quality, food availability, and reduced number of predators (Iwama et al., 1997). The annual gonadal cycle of any fish depends on the external signals received from the environment. Water temperature and photoperiod are the most important environmental factors, influencing the reproductive functions of fish (Lam, 1983; Lamba et al., 1983; MacKenzie et al., 1989; Peter and Yu, 1997). Rain has described as another important factor in regulating the reproductive function (Lamba et al., 1983; Gentile et al., 1986; Singh and Singh, 1987; Machado-Allison, 1992, 1994; Marcano et al., 2007). The gonadal maturation is started a few

months before the beginning of the rainy season (Machado-Allison, 1987). Factors such as physical and chemical changes in the water (pH, conductivity, oxygen concentration, temperature), and the full availability of shelter, phytoplankton and zooplankton, larvae, seeds, etc., have been found to have variable effects on different species during the rainy season (Machado-Allison, 1987). In most teleost fish, the gonadal recrudescence process positively correlated with the progress of the dry season, in such a manner that the maximum maturational stage is achieved by the end of the dry season (Gonzalez, 1980; Gentile et al., 1986; Guerrero et al., 1990). The onset of the rainy season provides favorable environmental conditions, promotes spawning and spermiation. Breeding, which is completed in a very short time, is followed by a period of sexual quiescence during the rainy season. There are several studies on fish reproductive or teleost breeding (Clark, 1934; Yuen, 1955; Devaraj, 1973; Sobhana and Nair, 1974; Babiker and Ibrahim, 1979; Blay and Eyeson, 1982; Cyrus and Blaber, 1984; Bagarinao et al., 1986; Witthames and Walker, 1987; Choudhury et al., 1990; Hossain, et al., 1992; Koya et al., 1995; Begum, 1997; Kamisaka et al., 1999; Bhowmik et al., 2000; Sarkar et al., 2005; Leonardos et al., 2009; Banik et al., 2011; Banik and Bhattacharya, 2012).

3. Objectives

The reproductive responses depended on many natural and aquatic environmental factors. The present study provides effective knowledge for spawning pattern of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794). The objectives of the present work were as followed:

1. Exploration of morpho-metric and reproductive parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in different Indian major rives and their tributaries.
2. Annual reproductive cycle of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in Lucknow region.
3. Induced breeding performance of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) with different hormones.

The thesis is divided into 3 chapters, besides a general introduction, consolidated summary and conclusion. The chapters were written in research manuscript form and references of all chapters were compiled at the end of the thesis to avoid repetition.

Chapter I

Exploration of morpho-metric and reproductive parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in different Indian rivers

ABSTRACT

The present work was focused to explore the freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794) from wild population of different Indian rivers (Betwa, Brahmaputra, Cauvery, Chambal, Ganga, Ghaghara, Gomati, Hooghly, Krishna, Mahanadi, Narmada, Ramganga, Sharda, Sone, Subarnarekha, and Tapti). The exploration was performed in relation to morpho-metric and reproductive parameters assessment viz., body weight and depth, total length, fork length, standard length, gonadal weight, gonadosomatic index, ovarian protein, fecundity, oocyte weight and oocyte diameter. There was significant correlation between different studied parameters of explored Indian rivers ($p < 0.05$). The result found that River Narmada has best sample of *Ompok bimaculatus* in respect to body weight (95.8±18.8 gm) and depth (59.1±5.4 mm), total length (283.1±38.8 mm), fork length (256.4±35.1 mm), standard length (239.0±33.4 mm), gonadal weight (14.08±0.95), gonadosomatic index (14.79±0.004), ovarian protein (7.98±0.0003) and fecundity (21512.57±5606.06). However, oocyte weight was higher in Krishna River sample and oocyte diameter was higher in Cauvery River sample. Changes in gonadosomatic index (GSI), ovarian protein and ovarian

histology were also observed in preparatory, pre-spawning and spawning phase of reproductive cycle. GSI and ovarian protein concentration distributions were correlated significantly ($p < 0.05$) by linear regression analysis. The histological assessment showed that ovaries exhibited seven stages of oocyte development, which were oogonia, chromatin nucleolar, early perinucleolar, yolk granules, late perinucleolar, vitellogenesis and vitellogenic oocyte stages in all the three phases of reproduction. The observation of fish sampled from different Indian rivers showed that Narmada River has better adaptive condition for growth and breeding of *O. bimaculatus* in comparison of others rivers.

Keywords: Ompok bimaculatus, morpho-metric, reproductive parameters, Indian Rivers.

1. Introduction

Rivers have played a critical role in the growth of human civilizations across the globe. Humans have interacted with rivers and their floodplains. Rivers are well-known habitat for fish. Very few studies have been made on the ecology in relation to the Riverine fisheries (Singh et al., 1988; Biswas and Michael, 1992; Biswas et al., 1995; Biswas and Boruah, 2000a, b). The total length of Rivers in India is about 29,000 km. In India, the rivers support rich biodiversity and offer livelihood and nutritional security but unfortunately this has been less studied from conservation planning and management point of view. Although, studies on the fish fauna of the River Ganges and its tributaries have been made by several authors and information was mostly reported on the systematic, bio-geographical and ecological aspects (Hamilton, 1822; Day, 1875-1878; Bilgrami and Munshi, 1985; Krishnamurti et al., 1991; Payne et al., 2004; Sarkar et al., 2010) but these information are still inadequate to address the critical issues related to the conservation of fish.

Today fish diversity and associated reproductive biology are a great challenge (Dudgeon et al., 2006). There is a lack of information related to reproductive aspects of near threatened fish and its conservation and management planning except few (Payne et al., 2004; Sarkar et al., 2008a, 2010). In order to correlate the environmental health of the

ecosystem of different Indian Rivers, *Ompok bimaculatus* fish species was monitored for different ecological exposure and biological effects by measuring a suite of biological responses on biological scales as a morpho-metric analysis and reproductive outcomes.

Morphometry (Gk: morphe: shape or form; metria: measurement) is one of the commonly used method (Sajina et al., 2011), which refers to quantitative analysis of forms, i.e., size and shape (Anonymous, 2015). It has been used not only to differentiate taxonomic units but the variations of its features are probably related to the habit and habitat among the variants in this species (Ambily and Nandan, 2010). It gives substantial information with regard to exact identification key of the species (Cavalcanti et al., 1999). Morpho-metric characterization may also be able to provide conceptual links between morphology and the genetic, developmental and evolutionary processes and factors that influence it (Sajina et al., 2011; Anonymous, 2015). Fish morphology is significantly influenced by environmental conditions (Szczyglinska, 1980a, b; McLaughlin and Grant, 1994; Brinsmead and Fox, 2002; Neat et al., 2003). A number of authors working on fish morphology attempted to find the possible influence on the body ratio and morphological features in individuals of fish from different habitats (Taylor, 1986; Dynes et al., 1999; Von Cramon Taubadel et al., 2005; Sacotte and Magnan, 2006).

Another important parameter is length-weight relationship which was first time described by Huxley (1924) as the relationship between length and weight. The power function, suggested by him, has proved to be a useful model for weight as function of length (Anderson and Gutreuter, 1983). Pauly (1983) reported the importance of length-weight relationship in the calculation of an equation of growth in length into an equation of growth in weight. Whereas Arslan et al. (2004) stated that it is usually easier to measure length than weight and weight can be predicted later on using the length-weight relationship. Length-weight relationships are major tools for precise estimation of biomass and calculation of length frequency (Petrakis and Stergiou, 1995; Pauly and Gayanilo, 1996). The concise relationship between body weight and length is always a unique; different among species of fish and even fish of same species and this reflects innate, specific, robustness of fish and inherited body shape configuration (Bayley,

1991). It is also an essential component of morphological and statistical analysis of fish growth, length and age ecological patterns and such other population structures (Kohler et al., 1995). It is also used to evaluate the relative condition of fish (Le Cren, 1951; Bagenal and Tesch, 1978; Petrakis and Stergious, 1995) and to understand the biological changes in fish stocks (Le Cren, 1951). It also used to know about the reproductive history or life history of fish and morphological comparisons of population from different regions (Wooton, 1990; Wootton, 1992; Petrakis and Stergiou, 1995).

Besides the morpho-metric study tools, the main aspects that comprise the reproductive strategy of fish species are gonadosomatic index (GSI), ovary weight, ovarian protein concentration, fecundity, oocyte diameter, oocyte weight and ovarian morphology. Understanding these aspects can be considered as the first step in establishing the principal life-history patterns of fish species and in determining the suitability of geographic and aquatic ecological area (Matthews, 1998; Mazzoni and Silva, 2006). This will help in observing the environmental stress and long term negative effects on population. Fecundity, GSI and ovarian protein level are important to estimate the reproductive potential of a fish species in relation to their size and environmental conditions in which they live (Bagenal and Braum, 1978; Fawole and Arawomo, 2000; Brown-Peterson et al., 2002).

The gonadosomatic index (GSI) is one of the important parameters of the fish biology, expresses either gonadal recrudescence or gonad growth from the regressed state to full maturity (Sathyanesan, 1959; Belsare, 1962; Lehri 1968; Siddiqui, 1977; Saksena, 1987; Shashi and Akela, 1996; Rao et al., 1999; Mohan and Jhajhria, 2001; Gupta and Shrivastava, 2001; Hojo et al., 2004; Shankar and Kulkarni, 2005). Hogg (1976) reported that GSI values are better observed in the rainy season which is the peak spawning season. It measures the cyclic changes in gonadal weight in relation to total fish weight (Ahirrao, 2002).

The fecundity of fish is described as seasonal spawning potential and alternatively is defined as the number of ripe eggs between current and next spawning period in a

female (Bagenal and Tesch, 1978). The egg production varies not only among different species but also within the same species depending upon the length and weight of gonad, influenced by the environment or geographical distribution (Somvanshi, 1985; Kulshrestha et al., 1990; Barmanh and Saikia, 1995; Shinde et al., 2002). Nikolskii (1969) indicated that the success on reproductive fertility of fecundity may vary depending on the food supply nature and differences, temperature, environmental circumstances, stress condition, fish size and length or even due to density-dependent mechanisms which are authenticated by Tsai and Gibson (1971), Sztramko and Teleki (1977), Bagenal (1978b) and Treasurer (1981). Moreover, smaller fish contains small visceral space and ovaries for eggs compared to large fish and therefore, some studies indicates that egg development and size related to fish body length and sometime on the environmental condition (Bagenal and Tesch, 1978; Hislop, 1988; Wright and Shoesmith, 1988; Moyle and Cech, 2000). Consequently, scientist generally uses the fecundity information to better understand the relationship between the survival species and its environment (Iversen, 1996). Recent work on GSI and fecundity was done by several workers on different fish (Abedi et al., 2011; Hossain et al., 2012; Isa et al., 2012; Mishra and Saksena, 2012; Gupta and Banerjee, 2013; Kasheif-El et al., 2013; Nandikeshwari and Anandan, 2013).

The reproductive organs possess a series of developmental changes which are closely accompanied by conspicuous cellular, biochemical, molecular and endocrinological changes (Nagahama, 1983; Guraya, 2000). The fish protein are easily digested and assimilated. It is mostly incorporated in to muscles of fish. In the breeding season, the gonads increase in size; somatic growth slow down and eventually stop. At this stage proteins of somatic tissue are transferred to the reproductive organs (Aksnes et al., 1986; Chandrasekhara and Krishnan, 2011). The ovarian morphology show signs of interaction between pollution and physiological processes (Depledge and Fossi, 1994; Ham et al., 1997; Walker, 1998; Adams et al., 1999). The ovarian developmental study is very vital for understanding the fish culture and conservation.

Ovarian development occurs in three phases which are the primary growth phase, secondary growth phase and maturation phase (Coward and Bromage, 1998; Cek et al., 2001). There are five methods used to determine the gonadal developmental stages of fish, which may differ according to their precision, cost and processing time: (1) Histological analysis which is the most accurate and effective for increasing efficiency of levels of performance ovulation and reproductive pattern (Delahunty and DeVlaming, 1980) (2) Oocyte size measurement using light microscope: effectively identifies the most advanced mode. The most important thing is that the numbers of oocytes measured provides acceptably precise estimates (West, 1990) (3) Microscopic classification of whole oocytes (4) Macroscopic staging which is a quick and gross method (West, 1990) (5) GSI determination which is the least laborious (West, 1990).

The biological aspects like morpho-metric parameter and length weight relationship, and reproductive parameters viz., gonadosomatic index, gonadal weight, ovarian protein level, fecundity, oocyte diameter and oocyte weight play a very important role in fish conservation. Information about freshwater butter catfish, *Ompok bimaculatus* is scarce. Thus the present study has been undertaken for the first time to generate the comprehensive and comparative account of the morpho-metric and reproductive outcomes of freshwater butter catfish, *Ompok bimaculatus* between the different explored Indian rivers which could be useful in defining habitat, geographical and ecological situations.

2. Materials and Methods

2.1. Chemicals

All the chemicals used in the sample collection, various observations and estimations were of analytical grade, and purchased locally from scientific suppliers, Lucknow, Uttar Pradesh, India.

2.2. Preliminary sites of sampling fish

The freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794) were collected from different wild populations of Indian rivers (viz., Betwa, Brahmaputra, Cauvery, Chambal, Ganga, Ghaghara, Godavari, Gomati, Hooghly, Krishna, Mahanadi, Narmada, Ramganga, Sharda, Sone, Subernrekha and Tapti). The fish sampling sites and GPS coordinates of different explored Indian rivers shown in Table 1 and Figure 1a-1. These rivers are distributed in four climatic zones based on the rainfall viz., heavy rainfall with more or less moderate temperature, heavy seasonal rainfall with persistent high temperature, indiscrete rainfall with fluctuating temperature in summer and winter, very low rainfall with high temperature fluctuation in summer.

2.3. Fish sample collection

The fish were handled in accordance with local/national guidelines for experimentation on animals and all care was taken to prevent cruelty of any kind.

The morning period was selected for sampling. The fish samples were collected from gill net and drag net at different sites. The fresh specimen was selected for the morpho-metric parameter analysis. The morpho-metric parameters viz., body weight and depth, total length, standard length and fork length (Figure 2) were recorded at the sampling sites. For reproductive parameters analysis, fish sampled from different sites were dissected out to collect ovary. The body and ovarian weight was taken with help of digital electronic balance (Smart Aqua Series: 0.01 accuracy) before the further examinations. The 5-10 ovarian tissues were kept in a chilled-box properly to bring to the laboratory for protein estimation and some tissues were also fixed in Bouin's solution for histological purpose.

After 8 hr of fixing in Bouin's solution, the ovarian tissue was trimmed in to small size sections. Further for remaining 16 hr, the tissue was then fixed in Bouin's solution. After 24 hr of fixtation, the tissues were fixed into 70% alcohol. For the estimation of fecundity and oocyte diameter, the ovary of minimum of 10-15 fish collected from different sites were taken out and preserved in 5% formalin.

2.4. Study of reproductive parameters

The rest of the experiments related to the reproductive parameters assessment of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) from 16 Indian rivers was carried out in the laboratory of Department of Applied Animal Sciences, School of Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow.

To see the seasonal variation in reproductive parameters viz., gonadosomatic index, ovarian protein concentrations and ovarian anatomy of freshwater catfish, *Ompok bimaculatus* (Bloch, 1794) in different reproductive phases (preparatory, pre-spawning and spawning phase), nine Indian major rivers (Brahmaputra, Cauvery, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subernrekha, Tapti) were selected.

2.4.1. Gonadosomatic index (GSI)

To understand the reproductive condition of fish, the gonadosomatic indices were measured. The gonadosomatic index (GSI) was calculated according to Lagler (1956) as:

GSI= weight of gonad x 100/ weight of fish

2.4.2. Length –weight relationship

The various size of freshwater butter catfish, *Ompok bimaculatus* samples were collected from different explored Indian rivers representing different habitats. The sampled fish individuals were measured for total length and body weight (determined to the nearest 0.01 g using digital electronic balance (Smart Aqua Series: 0.01 accuracy). The length- weight relation for female fish was calculated by using the standard equation of Huxley (1924):

$$W=a\,L^{b}$$

This can be expressed in logarithmic form as

Log W=log a + b (log L) suggested by Le Cren (1951)

where,

W=weight of the fish in g;

L=length of the fish in mm

a=intercept; b=regression co-efficient or slope of the line (exponent value).

2.4.3. Estimation of ovarian protein concentration

The ovarian protein concentration was estimated by the method of Lowry et al., (1951) method using crystalline Bovine Serum Albumen (BSA) as standard with a spectrophotometer (UV-Thermo). The procedure of estimatuion was given below in the perspective sections. The concentration was calculated by using the following formula:

Protein concentration= O. D. of unknown / O.D. of known × 100

2.4.3.1. Preparation of Reagents

- Reagent C (alkaline copper sulphate reagent): 50 ml reagent A and 1 ml reagent B
- Reagent A (sodium carbonate 2%): 2 gm of sodium carbonate and 100 ml of (0.1 N) NaOH (0.1 N NaOH=0.4 gm of NaOH and 100 ml distilled water)
- Reagent B (copper sulphate 0.5 %): 0.5 gm copper sulphate and 100 ml of sodium potassium tartarate (1.35%)
- Sodium potassium tartarate (1.35%)=1.35 gm of sodium potassium tartarate and 100 ml distilled water.

2.4.3.2. Procedure of standard preparation

Different concentrations (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml) of standard solution (0.02 gm Bovine Serum Albumin: BSA and 50 ml 0.1 N NaOH) was taken in 6 test tubes separately.

↓

Each was then made up to 1 ml with distilled water.

↓

5 ml of alkaline copper sulphate reagent (reagent C) was added to each. mixed well and allowed to stand at room temperature for 10 minutes.

↓

0.5 ml of 1 N Folin-Ciocalteu reagent was added: left to stand undisturbed for 20 minutes. Appearance of blue colour indicated presence of protein.

2.4.3.3. Preparation of ovarian sample

The ovarian tissues were homogenized in 1ml PBS (Phosphate buffer saline) and centrifuged at 5000 rpm for 20 minutes.

↓

50 µl supernatant of the sample was added with 0.95 ml of distilled water

↓

Added 5 ml of alkaline copper sulphate

↓

Allowed to stand for 10 minutes at room temperature

↓

0.5 ml of 1 N Folin-Ciocalteu reagent was added to the solution and left to stand for 20 minutes at room temperature.

↓

Appearance of blue colour indicated presence of protein

2.4.3.4. Procedure for preparation of Blank

0.5 ml of distilled water was taken in a test tube

↓

added 0.5 ml of NaOH

↓

then added 5 ml of alkaline copper sulphate (reagent C) and left to stand for 10 minutes at room temperature

↓

0.5 ml of 1 N Folin-Ciocalteu reagent was added to it and left to stand for 20 minutes at room temperature

↓

Standard solution and samples were run at 650 nm wavelength through the spectrophotometer (Thermo electron carporation UV-1) against a reagent blank and measured standard solution and sample absorbance of ovarian tissue.

2.4.4. Fecundity

For the estimation of fecundity, only mature ova were taken into consideration. The ovaries were taken out from formalin solution (5%) and placed between the fold of blotting paper. A total of 5 ripe ovaries of fish species of each explored Indian rivers were selected. Gravimetric method or weight method (Lagler, 1956) was used for estimation of the fecundity of *O. bimaculatus*. Gravimetric method seemed to offer the best possibility of minimizing error due to its simple and easy sampling techniques. The Gravimetric or weight method has been successfully used by Doha and Hye (1970), Shafi and Quddus (1974), Dewan and Doha (1979), Mustafa et al. (1983). The gravimetric method was done by the following way:

a) Ovaries were cut into some sections or pieces from anterior, middle and posterior regions of each ovarian lobe
b) Weighed adjusted to 0.2 gm each.
c) Each sub-samples of ovary was spread over slide.
d) Observe under the binocculer (2X) olympus.
e) One by one count these eggs of whole slide.

This value was proportional to the total ovary weight; the number of eggs (F1) for the sub-sample was estimated by using the following equation (Yelden and Avsar, 2000):

Fecundity (F1)=No. of eggs in the ovary sample x Gonad weight / Ovary sample weight

Later, by taking the mean number of three sub-sample fecundities (F1, F2 and F3), the individual fecundity for each female fish was calculated by the following equation:

Fecundity (Fe)=(F1 + F2 +F3) / 3

2.4.5. Oocyte diameter

To find out the oocyte diameter, 10 ova were taken randomly from the preserved sample of ovaries of three portions of each ovary (anterior, middle and posterior region). These samples were spread uniformly over a glass slide. The diameters of ova were measured in a straight line under compound microscope at 10X magnification using stage and eye-ocular micrometer. One micro-meter division (m.d.) of the ocular micrometer was equal to 0.016 mm.

2.4.6. Ovarian histological processing

The fixed tissues were processed for further process of histology. The procedure of histology from fixation to mounting with DPX was given in Table 2. The microscopic developmental stages of oocyte were categorized according to Janssen et al. (1995).

2.5. Statistical analysis

Data were expressed as the mean±SEM. Significance for each parameter in different Indian rivers was checked by one way analysis of variance (ANOVA) at $p < 0.001$, followed by multiple comparison with Newman-Keuls test $p < 0.05$ in order to see the differences between the locations. To analyze interdependency of the parameters, Pearson correlations, was done by using software IBM SPSS (version 20.0). The data were also subjected to regression analysis and coefficient of correlation (r^2).

Table 1: The different exploration sites of Indian rivers for collection of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794)

Rivers	**Sites**	**GPS Coordinates**
Betwa	Jhansi	N 25°26′55″ E 78°34′11″
Brahmaputra	Guwahati	N 25º13'24" E 89º41'41"
Cauvery	Mysore	N 11°21 ′40″ E79°49′46
Chambal	Gwalior	N 26°29′20″ E 79°15′10″
Ganga	Farakka	N 24°48'58" E 87°55'55"
Ghaghara	Faizabad	N 26°46′07″ E 82°08′06″
Godavari	Nirmal	N 19°55′48″ E 73°31′39″
Gomati	Lucknow	N 25°30′29″ E 83°10′11″
Hooghly	Naihati	N 22°53'19" E 88°25'22"
Krishna	Vijaywada	N 17°55′28″ E 73°39′36″
Mahanadi	Bhubaneswar	N 9°11′50″ E 99°22′57″
Narmada	Hosangabad	N 21º39'3.77" E 72º48'42.8"
Ramganga	Bareily	N 26°28′21″ E 80°19′52″
Sharda	Pallia	N 28 º16.043' E 08103.991'
Sone	Rewa, Rihand	N 25°42′9″ E 84°51′54″
Subernrekha	Ranchi	N 21°33′18″ E 87°23′31″
Tapti	Surat	N 21º14'53.67"E 73º35'21.87"

Table 2: Detailed procedure of histology (fixation, staining, dehydration and mounting) with its time interval

Steps	**Descriptions**					**Time interval**
Fixation of Tissues	Fixed in Bouin's fixative					24 hr
	Washing in tap water and then distilled water					15 min. each (2 times)
Dehydration	30% alcohol	50% alcohol	70% alcohol	90% alcohol	100% alcohol	30 min. in each
Clearing	Xylene + absolute alcohol (1:1)		Xylene			15 min. in each
Embedding	Xylene + wax (1:1)	Wax_1	Wax_2	Wax_3		15 min. in first step 30 min. for further (2 times each)
Block preparation	Now tissue were placed in the metal L shaped angle kept ready by filling with wax and the air bubble arising were removed by using hot spatula.					
Trimming	The excess of wax was trimmed out till the material was slightly visible and then it was fixed on the block holder for sectioning.					
Sectioning	Sectioning was performed on rotary microtome (Weswox). The sections were of 5µm thickness.					
Mounting	With Mayer's egg albumin; slides were put on hot plate having approximately 30-35^{0}C temperature.					
Deparaffinised	$Xylene_1$	$Xylene_2$	Xylene + absolute alcohol			5 min. (each)

			(1:1)			
Hydration	100% alcohol	90% alcohol	70% alcohol	50% alcohol	30% alcohol	10 min. (each)
Washing	Distilled water		Tap water			10 min. (each)
Staining	Haematoxylin	Tap water	Distilled water			5-10 min. (each)
Dehydration	30% alcohol	50% alcohol	70% alcohol			10 min. (each)
Staining	Eosin	90% alcohol (1-2 dip)	100% alcohol then Xylene + absolute alcohol (1:1)		2-5 min. in Eosin	2 min. (each)
Mounting	DPX					
Examination	The histological sections of gonadal tissues were examined under Bright field microscope (Olympus CX41) using micropublisher 3.3 RTV camera (Qimaging, BC, Canada) at 20X magnification.					

Figure 1a: Photographs of different sites of explored Indian rivers (Betwa, Brahmaputra and Cauvery Rivers) and sampled freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

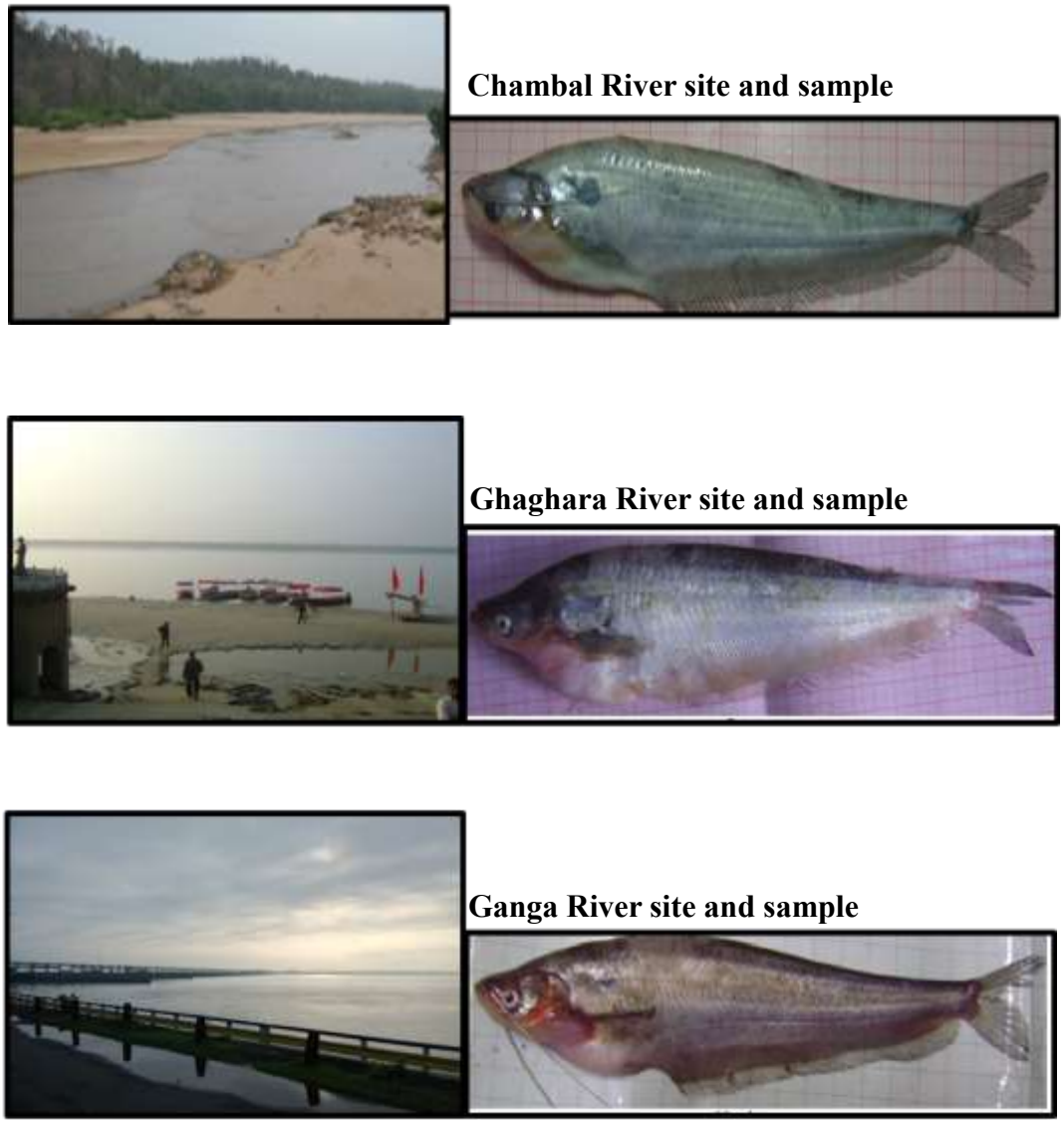

Figure 1b: Chambal, Ghaghara and Ganga Rivers with their sampled freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

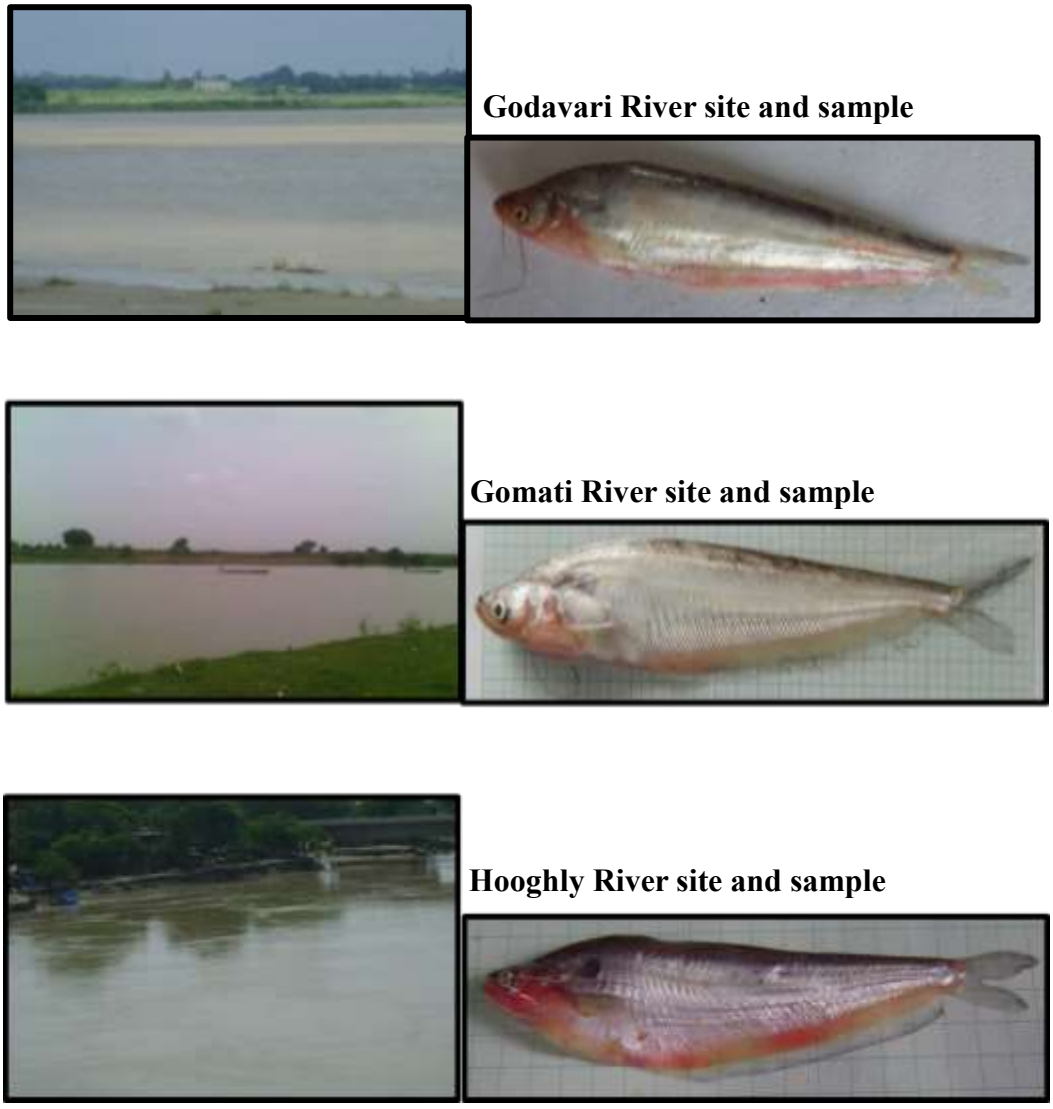

Figure 1c: Godavari, Gomati and Hooghly Rivers with their sampled freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

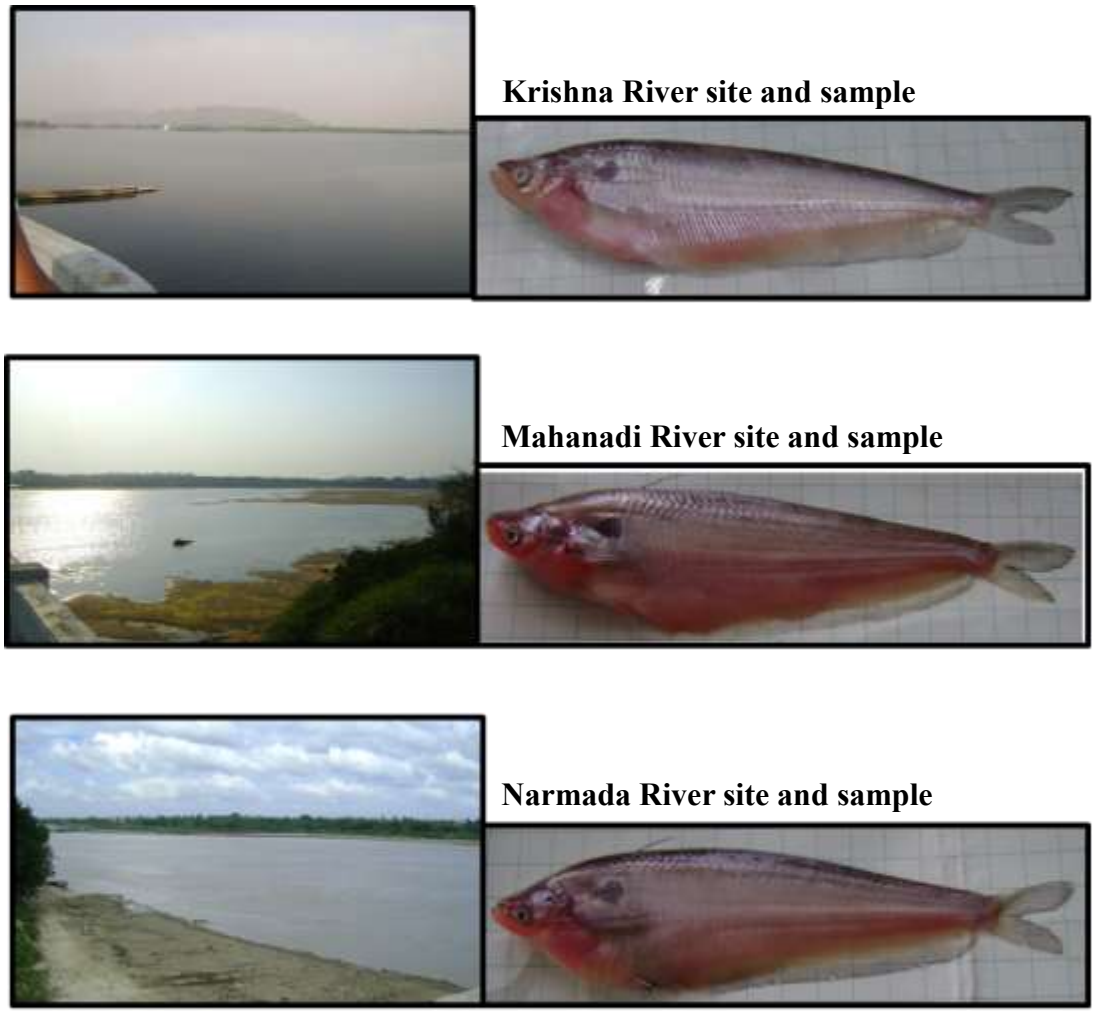

Figure 1d: Krishna, Mahanadi and Narmada Rivers with their sampled freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

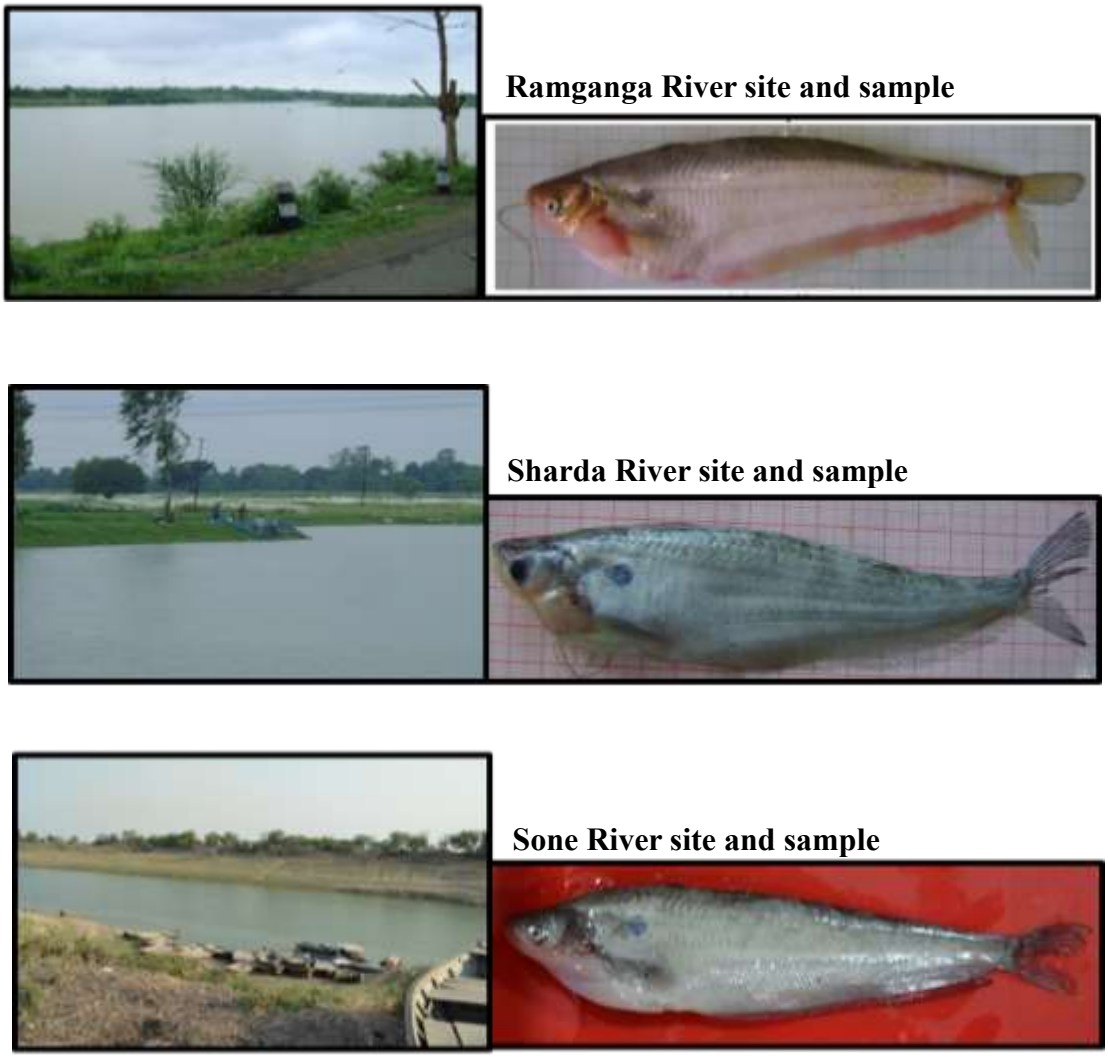

Figure 1e: Ramganga, Sharda and Sone Rivers with their sampled freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

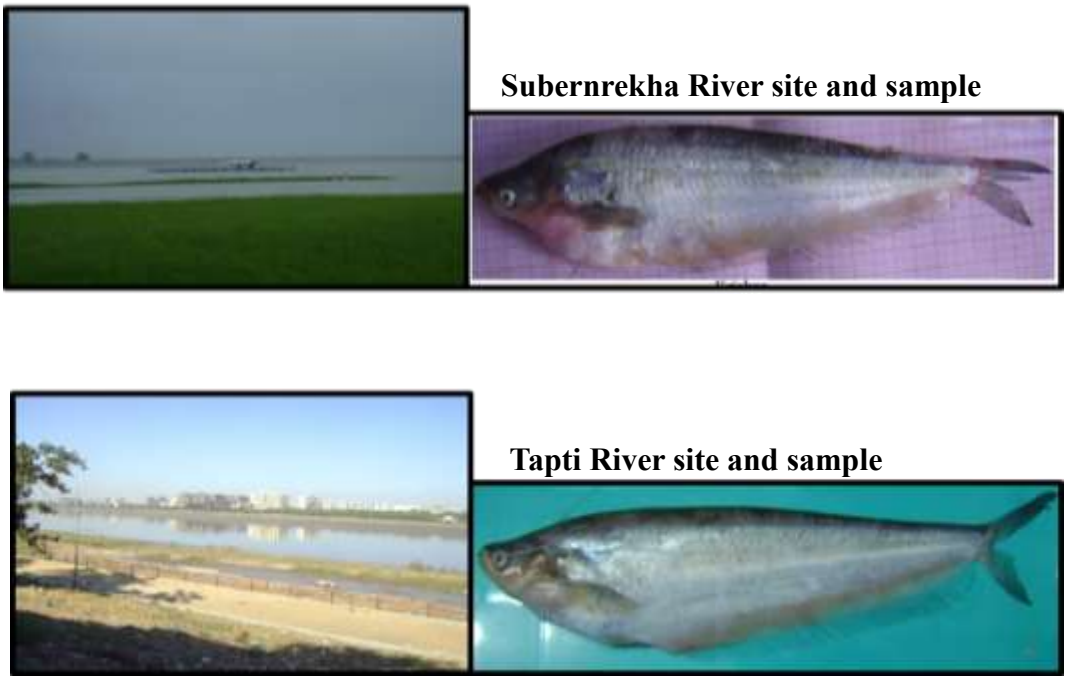

Figure 1f: Subernrekha and Tapti Rivers with their sampled freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

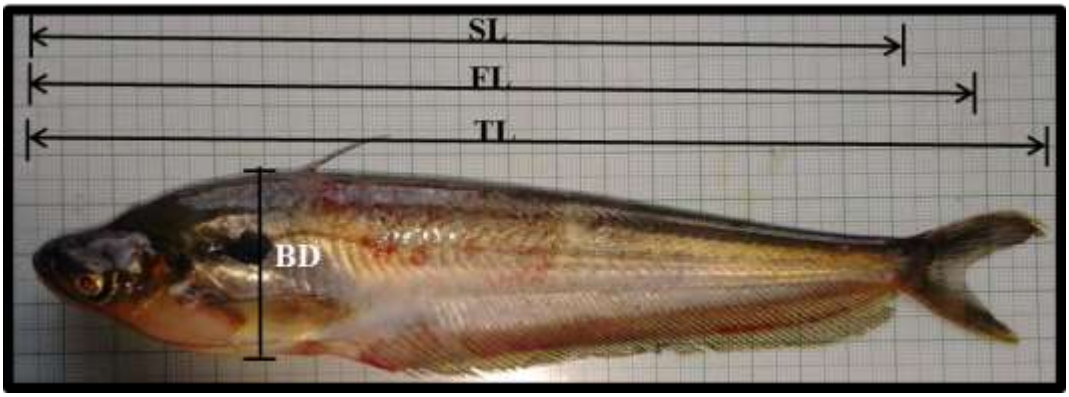

Figure 2: Photograph of experimental freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) showing the various observed morpho-metric parameters viz., SL: Standard length, FL: Fork length, TL: Total length, BD: body depth.

3. Result

3.1. Morpho-metric assessment of freshwater butter catfish, Ompok bimaculatus sampled from different explored Indian rivers

The observation of morpho-metric parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) resulted that there was found a wide range of body weight (BW) and body depth (BD) from different rivers (57.3±13.2 to 95.8±18.8 gm: BW and 38±8.6 to 59.1±5.4 mm: BD). The highest body weight and depth of fish was measured in the sample of Narmada River (95.8±18.8 gm: BW and 59.1±5.4 mm: BD) and lowest body weight and depth fish was recorded in Hooghly River sample of freshwater butter catfish *O. bimaculatus* (57.3±13.2gm: BW and 38±8.6mm: BD) (Figure 3A). The BW and BD showed a significant difference among different explored Indian river for freshwater butter catfish *Ompok bimaculatus* (F=1086.67 for BW and F=1081.72 for BD at $p < 0.001$).

The other morpho-metric parameters such as total length (TL), fork length (FL) and standard length (SL) were also observed in the model fish *O. bimaculatus* sampled from wild population of Indian rivers. The observed reproductive parameters (TL, FL and SL) showed a significant difference among different explored rivers of India for freshwater butter catfish *O. bimaculatus* (F=1427.54 for TL; F=1595.46 for FL; F=1410.42 for SL at $p < 0.001$). The result showed that Narmada River had the best species in respect to its TL, FL and SL. It had highest total length (283.1±38.8 mm), fork length (256.4±35.1 mm) and standard length (239.0±33.4 mm) as compared to other sampled fish from explored rivers. The fish sample of lowest TL (192.6±31.4 mm), FL (173.9±28.4 mm) and SL (166.1±26.1 mm) was noticed from Hooghly river sample (Figure 3B) and the rest of explored river sample of *O. bimaculatus* came between those highest and lowest value of body weight and depth, total length, fork length and standard length (Figure 3A and B).

3.2. Reproductive parameters assessment of freshwater butter catfish, Ompok bimaculatus sampled from different Indian rivers

The reproductive parameters such as gonadosomatic index (GSI), gonadal weight (GW), fecundity, ovarian protein concentration (OPC), oocyte weight (OW) and oocyte diameter (OD) of *O. bimaculatus* was significantly different among different explored Indian rivers (F=350.46 of GSI; F=422.6 for GW; F=185.58 for fecundity; F=227.83 for OPC). However OW and OD of *O. bimaculatus* was not significantly different (F=15.63 for OW; F=27.43 for OD). The GSI of the studied fish sampled from different Indian rivers, was varied from 2.35 to 14.79. It was ranged highest for Narmada river (14.79±0.004) and lowest for Mahanadi river (2.35±0.005). A similar pattern was observed for gonadal weight (mg) with highest gonadal weight (14.08±0.95 mg) for Narmada river and lowest for Mahanadi river (2.1±0.35 mg) (Figure 4A).

The ovarian protein level of *O. bimaculatus* was varied with different wild population of explored rivers. It was ranged from 3.6 to 7.98 mg/ml/100mg tissue weight. The highest ovarian protein concentration was recorded for river Narmada (7.98±0.0003 mg/ml/100mg tissue weight) and lowest from the river Ramganga (3.6±0.0024 mg/ml/100mg tissue weight) (Figure 4B). It was positively correlated with fecundity. The observed range of fecundity was varied from 5293.23 to 21512.57. It was ranged highest for river Narmada (21512.57±5606.06) and lowest for river Mahanadi (5293.23±1676.96) (Figure 5A).

The observations of oocyte diameter of *O. bimaculatus* surveyed from wild population among Indian rivers presented the range from 0.21 to 0.34 mm. It was noticed that sampled collected from Cauvery river have a higher diameter (0.34±0.04 mm) and lowest from Mahanadi (0.21±0.02 mm) (Figure 5B). The oocyte weight was analyzed highest in major river Krishna (0.58±0.11 mg) and lowest from river Godavari (0.28±0.06 mg) (Figure 5B).

3.3. Correlation between morpho-metric and reproductive parameters of freshwater butter catfish Ompok bimaculatus

The correlations between different studied morpho-metric and reproductive parameters of *O. bimaculatus* sampled from explored Indian rivers were noticed by Pearson correlation. The correlation (r) and coefficient of correlation (r^2) was given in the Table 3 and 4. The result showed that the highest correlation was exist between fork length-standard length (FL-SL), fork length-total length (FL-TL), total length-standard length (TL-SL), body depth-fork length (BD-FL), BD-SL, body weight-total length (BW-TL), BW-SL, BD-TL, BW-BD, ovarian protein concentration-fecundity (OPC-FEC) and gonadal weight-gonadosomatic index (GW-GSI) ($r > 0.624$; $p < 0.01$). The correlation of body depth (BD) versus FL, SL and TL was 0.939, 0.925 and 0.898 at $p < 0.01$ (Table 3). Maximum correlation observed in Fork length- Standard length (FL-SL) ($r=0.994$; $p < 0.01$) and minimum correlation was noticed in body weight-standard length (BW-SL) ($r=0.624$; $p < 0.01$, Table 3). The moderate correlation was recorded between BW-FL, GW-FL, GW-SL, GW-BD, FEC-GW, FEC-GSI, OPC-GW, OPC-GSI ($r > 0.525$; $p < 0.05$, Table 3). The least correlation was recorded between BW-OPC, BW-OD, BW-GW, BW-GSI, TL-FEC, TL-OPC, TL-OD, TL-GW, TL-GSI, FL-FEC, FL-OPC, FL-OD, FL-GSI, SL-FEC, SL-OPC, SL-OD, SL-GSI, BD-FEC, BD-OPC, BD-OD, BD-OW, BD-GSI, FEC-OW, FEC-OD, OPC-OW, OW-OD, OW-GSI, OD-GW and OD-GSI ($r < 0.525$; Table 3). Some negative correlations were also found in BW-FEC, OPC-OD, OW-BW, OW-FL, OW-TL, OW-SL and OW-GW (Table 3). Hence from the result of correlation, it was concluded that body weight of fish appeared to increase with increase in body depth, total length, fork length, standard length, gonadal weight, oocyte diameter and weight, gonadosomatic index. Equation of regression showed linear line relationship between those studied morpho-metric and reproductive parameters (Table 4).

3.4. Length –Weight relationship

Length-weight relationships of female *Ompok bimaculatus* (Bloch, 1794) sampled from different wild population of Indian rivers viz., Betwa, Brahmaputra, Cauvery,

Chambal, Ganga, Ghaghara, Gomati, Hooghly, Krishna, Mahanadi, Narmada, Ramganga, Sharda, Sone, Subernrekha and Tapti, were presented in Table 5. Scatter diagrams of relationship of length and weight had been plotted separately for different rivers (Figure 6-8). The isometric relationship was observed only in the fish samples of Betwa River and Mahanadi River. River Brahmaputra, Cauvery, Chambal, Ghaghara, Gomati, Narmada, Sharda, Sone and Tapti rivers showed negative allometric growth whereas River Subernrekha, Ramganga, Krishna, and Hooghly showed positive allometric growth. All relationships were highly significant ($p < 0.001$), with values of $r > 0.95$.

3.5. Seasonal study

To conduct seasonal study ,fish sampling was done from nine Indian major rivers of India (viz., Brahmaputra, Cauvery, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subernrekha and Tapti Rivers) in three different reproductive phases viz., preparatory, pre-spawning and spawning to study the seasonal variations in their gonadosomatic index, ovarian protein concentration and gonadal development through histology. Due to difference in their local habitat i.e. river conditions, wild population of *O. bimaculatus* had difference at their ovarian protein concentration and their fecundity as well. The obtained results showed that the freshwater butter catfish, *Ompok bimaculatus* was more or less registered same type of developmental stages in different wild population of Indian rivers.

3.5.1. GSI and ovarian protein concentration among different Indian rivers:

The result predicted that GSI of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different explored Indian major rivers was significantly different in different reproductive season (preparatory phase: F=125, pre-spawning phase: F=25 and spawning phase: F=89616.67 at $p < 0.001$; Figure 9, 10). It was ranged from 0.12±0.17 to 0.33±0.02 in preparatory phase, 0.34±0.04 to 0.48±0.01 in pre-spawning phase and 2.35±0.005 to 14.79±0.004 in spawning phase of reproduction. The result found that the sampled collected from Ganga river had high GSI in preparatory season

whereas samples of Narmada River had high GSI in pre-spawning and spawning phase (Figure 9A and B, 10). The lowest GSI was displayed in the sample of Tapti River in preparatory phase and Godavari River in pre-spawning phase and Mahanadi River in spawning phase.

The ovarian protein concentration of experimental fish sampled from wild population of Indian rivers was varied in respect to rivers and with seasons as well. The ovarian protein was varied from 1.05±0.0067 to 2.85±0.0063 mg/ml/100mg tissue weight in preparatory phase, 3.1±0.0003 to 3.96±0.001 mg/ml/100mg tissue weight in pre-spawning phase and 4.35±0.0015 to 7.98±0.0003 mg/ml/100mg tissue weight in spawning phase). Highest protein concentration was observed from Ganga (in preparatory phase) and from Narmada (in pre-spawning and spawning phase). Protein concentrations were low in case of Mahanadi and Godavari as in case of GSI. It showed a significant difference among reproductive phases and sampling areas (Figure. 9A and B, 10).

3.5.2. Relationship between GSI vs ovarian protein concentration in preparatory, prespawning and spawning phase

The Pearson's correlation relationship between gonadosomatic index and ovarian protein concentration in preparatory, pre-spawning and spawning phase was found to be significantly linear. The correlation (r) values were 0.917, 0.625 and 0.982 in preparatory, pre-spawning and spawning phase respectively. The linear regression equation was y=0.125x+(-0.025) for preparatory phase, y=0.2x+(-0.1) for pre-spawning phase and y=3.5x+(-12) for spawning phase (Figure 11). The linear relationship reflected that on increase in GSI of sampled fish *O. bimaculatus,* ovarian protein content was increasing in their respective phase. The correlation coefficient (r^2) between these parameters in preparatory, pre-spawning and spawning phase was (r^2=0.84; r^2=0.391; r^2=0.965) found to be highly significant at $p < 0.001$ level for preparatory, pre-spawning and spawning phase (Table 6).

3.5.3. Comparison of follicle stages among different explored Rivers

According to the development and maturity stages, the size, colour and turgidity of ovaries were changed. Macroscopic analysis showed that the resting ovaries were presented in small sizes and a gelatinous-semi-transparent appearance, while advanced maturing and mature ovaries had a vascularized dark brownish one (Table 7). The ovaries of preparatory phase were grossly characterized as two lobed, small sizes, slightly pinkish and transparent. Histologically ovary contained ogonium and peri-nucleus stage that stained basophilic in this season. Some small yolk vesicles were also seen. These early oocytes were contained a larger central nucleus. Nucleus had migrated to periphery and become eosinophilic. The histological sections of ovary represented that the most of the oocytes were in early peri-nucleus follicle stage, whereas the late peri-nucleus follicle stages were also distinguishably observed in few of the Rivers viz., Narmada, Tapti and Mahanadi river (Table 7; Figure 12, 15). At the maturing stage or pre-spawning phase, an increase in ovarian volume observed. It occupied one-fourth of the ventral cavity and increasingly vascularized. The oogonia were rounded and grouped and prominent nucleus was present. Most of the oogonia were basophilic in nature. Ovigerous folds and yolk vesicle were also seen. Germinal layer was going to become thin, the previtline cells were seen and oocyte was in oil drop stage and the nature of the oocyte was become slightly acidophilic. The histological sections showed large number of VI stage follicles that are in their late peri-nucleolus stage (Table 7; Figure: 13, 15). The histological sections of spawning phase oocyte had full of lipid yolk and protein yolk in centre. It pushed lipid yolk to the periphery. Dark blue colour rounded or hexagonal pre-vitellogenic oocytes were present. Oocytes were full of acidophilic yolk. Nucleus deformed, cytoplasm had abundant protein yolk globules. The vascular and thin ovarian wall was seen. Large mature oocyte with full of lipid yolk was noticed. Some of the oocytes showed germinal vesicle (Table: 7; Figure: 14, 15).

Table 3: Showing Pearson correlations between different morpho-metric and reproductive parameters (viz., body weight: BW, total length: TL, standard length: SL, fork length: FL, body depth: BD, fecundity: FEC, ovarian protein concentration: OPC, oocyte weight: OW, oocyte diameter: OD, Gonadal weight: GW and gonadosomatic index: GSI) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different explored Indian rivers. Asterisks (*) represented the significance level in Pearson correlation.

Correlations												
		BW	**TL**	**FL**	**SL**	**BD**	**FEC**	**OPC**	**OW**	**OD**	**GW**	**GSI**
BW	Pearson Correlation	1	.716**	.605*	.624**	.716**	-.044	.125	-.120	.021	.396	.038
	Sig. (2-tailed)		.002	.013	.010	.002	.873	.644	.658	.938	.129	.888
TL	Pearson Correlation	.716**	1	.955**	.953**	.898**	.167	.294	-.097	.225	.451	.195
	Sig. (2-tailed)	.002		.000	.000	.000	.536	.269	.720	.401	.080	.469
FL	Pearson Correlation	.605*	.955**	1	.994**	.939**	.338	.361	-.011	.319	.526*	.322
	Sig. (2-tailed)	.013	.000		.000	.000	.201	.170	.967	.229	.037	.224
SL	Pearson Correlation	.624**	.953**	.994**	1	.925**	.315	.347	-.052	.303	.540*	.328
	Sig. (2-tailed)	.010	.000	.000		.000	.235	.189	.848	.253	.031	.214
BD	Pearson Correlation	.716**	.898**	.939**	.925**	1	.339	.369	.005	.266	.571*	.333
	Sig. (2-tailed)	.002	.000	.000	.000		.200	.159	.986	.320	.021	.208
FEC	Pearson Correlation	-.044	.167	.338	.315	.339	1	.733**	.220	.008	.525*	.558*
	Sig. (2-tailed)	.873	.536	.201	.235	.200		.001	.412	.975	.037	.025
OPC	Pearson Correlation	.125	.294	.361	.347	.369	.733**	1	.282	-.206	.621*	.619*
	Sig. (2-tailed)	.644	.269	.170	.189	.159	.001		.290	.445	.010	.010
OW	Pearson Correlation	-.120	-.097	-.011	-.052	.005	.220	.282	1	.293	-.008	.033

Contd….

	Sig. (2-tailed)	.658	.720	.967	.848	.986	.412	.290		.270	.978	.905
OD	Pearson Correlation	.021	.225	.319	.303	.266	.008	-.206	.293	1	.116	.096
	Sig. (2-tailed)	.938	.401	.229	.253	.320	.975	.445	.270		.668	.722
GW	Pearson Correlation	.396	.451	.526*	.540*	.571*	.525*	.621*	-.008	.116	1	.926**
	Sig. (2-tailed)	.129	.080	.037	.031	.021	.037	.010	.978	.668		.000
GSI	Pearson Correlation	.038	.195	.322	.328	.333	.558*	.619*	.033	.096	.926**	1
	Sig. (2-tailed)	.888	.469	.224	.214	.208	.025	.010	.905	.722	.000	

****** Correlation is significant at the 0.01 level (2-tailed).

***** Correlation is significant at the 0.05 level (2-tailed).

Table 4: Showing coefficient of correlation (r^2) and regression line equation between morpho-metric and reproductive parameters (body weight: BW, total length: TL, standard length: SL, fork length: FL, body depth: BD, fecundity: FEC, ovarian protein concentration: OPC, oocyte weight: OW, oocyte diameter: OD, Gonadal weight: GW and gonadosomatic index: GSI) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different explored Indian rivers.

S. No.	**Studied Parameters**	**r^2**	**Regression line equation (y=Ax+B)**
1	BW-BD	0.513	y=1.583x+4.104
2	BW-TL	0.512	y=0.369x+(-3.691)
3	BW-FL	0.366	y=0.33x+12.006
4	BW-SL	0.389	y=0.381x+6.297
5	BW-GW	0.156	y=1.565x+67.795
6	BW-GSI	0.001	y=0.143x+77.959
7	BW-FECUNDITY	0.002	y=0x+81.356
8	BW-OD	0	y=7.214x+77.302
9	BW-OPC	0.016	y=1.229x+72.449
10	BW-OW	0.014	y=(-26.005)x+92.073
11	BD-TL	0.806	y=0.209x+0.372
12	BD-FL	0.882	y=0.232x+0.188
13	BD-SL	0.856	y=0.256x+0.256
14	BD-GW	0.326	y=1.023x+39.984
15	BD-GSI	0.111	y=0.564x+42.289
16	BD-FECUNDITY	0.115	y=0x+40.184
17	BD-OD	0.071	y=41.169x+36.192
18	BD-OPC	0.136	y=1.639x+38.374
19	BD-OW	0	y=0.454x+47.264
20	TL-FL	0.913	y=1.011x+18.695
21	TL-SL	0.907	y=1.130x+8.697
22	TL-GW	0.203	y=3.456x+199.667
23	TL-GSI	0.038	y=1.419x+212.011
24	TL-FECUNDITY	0.028	y=0.001x+209.611
25	TL-OD	0.051	y=149.815x+183.988
26	TL-OPC	0.086	y=5.598x+193.976
27	TL-OW	0.009	y=(-40.831)x+245.177
28	FL-SL	0.989	y=1.115x+(-9.345)

Contd….

29	FL-GW	0.276	y=3.817x+176.139
30	FL-GSI	0.104	y=2.214x+183.739
31	FL-FECUNDITY	0.114	y=0.002x+174.613
32	FL-OD	0.102	y=200.31x+149.184
33	FL-OPC	0.13	y=6.495x+168.023
34	FL-OW	0	y=(-4.502)x+206.358
35	SL-GW	0.292	y=3.499x+165.872
36	SL-GSI	0.108	y=2.014x+172.985
37	SL-FECUNDITY	0.099	y=0.002x+166.96
38	SL-OD	0.092	y=170.043x+144.88
39	SL-OPC	0.12	y=5.566x+160.6
40	SL-OW	0.069	y=(-18.44)x+200.614
41	GW-GSI	0.857	y=0.876x+(-0.739)
42	GW-FECUNDITY	0.275	y=0x+1.016
43	GW-OD	0.014	y=10.071x+4.574
44	GW-OPC	0.386	y=1.539x+(-1.219)
45	GW-OW	0	y=(-0.416)x+7.542
46	GSI-FECUNDITY	0.311	y=0x+2.117
47	GSI-OD	0.009	y=8.811x+6.799
48	GSI-OPC	0.384	y=1.622x+0.2
49	GSI-OW	0.001	y=1.883x+8.29
50	FECUNDITY-OD	0	y=956.33x+15481.74
51	FECUNDITY-OPC	0.538	y=2.375x+25.37
52	FECUNDITY-OW	0.049	y=15754.33x+7994.97
53	OD-OPC	0.042	y=(-0.006)x+0.307
54	OD-OW	0.086	y=0.186x+0.183
56	OW-OPC	0.08	y=0.013x+0.421

Table 5: Showing length weight relationship represent coefficients (a: intercept and b: slope) and r^2 for freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) collected from different explored Indian rivers.

S. No.	Rivers	Intercept, a	Slope, b	95%cl of a	95%cl of b	r^2
1	Betwa	0.72	2.99 (Isometric)	-5.57 to -4.93	2.86 to 3.12	0.968
2	Brahmaputra	0.66	2.69 (Allometric)	-4.77 to -4.41	2.61 to 2.77	0.989
3	Cauvery	0.52	2.21 (Allometric)	-3.75 to -2.95	2.02 to 2.35	0.972
4	Chamabal	0.59	2.43 (Allometric)	-4.31 to -4.05	2.25 to 2.58	0.961
5	Ganga	0.7	2.92 (Isometric)	-5.30 to -4.82	2.81 to 3.02	0.964
6	Ghaghara	0.63	2.59 (Allometric)	-4.53 to -4.15	2.51 to 2.67	0.966
7	Gomti	0.55	2.28 (Allometric)	-3.77 to -3.40	2.20 to 2.36	0.934
8	Hooghly	0.77	3.28 (Allometric)	-6.88 to -5.15	2.92 to 3.65	0.952
9	Krishna	0.78	3.29 (Allometric)	-6.68 to -5.59	3.04 to 3.53	0.984
10	Mahanadi	0.7	2.97 (Isometric)	-5.83 to -4.42	2.66 to 3.28	0.955
11	Narmada	0.22	1.44 (Allometric)	-1.87 to -1.51	1.36 to 1.52	0.984
12	Ramganga	0.82	3.55 (Allometric)	-6.89 to -6.40	3.45 to3.65	0.983
13	Sharda	0.67	2.73 (Allometric)	-4.95 to -4.51	2.63 to 2.82	0.973
14	Sone	0.53	2.25 (Allometric)	-3.83 to -3.02	2.08 to 2.42	0.971
15	Subernrekha	0.76	3.25 (Allometric)	-6.15 to -5.51	3.11 to 3.38	0.993
16	Tapti	0.48	2.07 (Allometric)	-3.41 to -2.66	1.91 to 2.23	0.968

(b=3: Isometric or ≠3: Allometric; Lower than 3: negative allometric and higher than 3: positive allometric)

Table 6: Showing Pearson correlations between gonadosomatic index (GSI), ovarian protein concentration and fecundity of freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794) of different Indian rivers. Asterisks (*) represented the significance level in Pearson correlation.

Season	**Correlations**		**Protein concentration**
Preparatory phase	GSI	Pearson Correlation	.917**
		Sig. (2-tailed)	.001
Pre-spawning phase	GSI	Pearson Correlation	.625*
		Sig. (2-tailed)	.072
Spawning phase	GSI	Pearson Correlation	.982**
		Sig. (2-tailed)	.000
** Correlation is significant at the 0.01 level (2-tailed).			
* Correlation is significant at the 0.05 level (2-tailed).			

Table 7: Showing the ovarian macroscopic and microscopic description of female freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

Ovarian phase	**Macroscopic appearance**	**Histological observation**
Preparatory	Ovary small in size, two lobed, slightly pinkish and transparent	Oogonia, early peri-nucleolus and chromatin nucleolus stage were seen.
Pre-spawning	Ovaries occupy one-fourth of the ventral cavity, reddish in colour and increasingly vascularised	Oocytes filled with yolk granule, vitellogenic and late peri-nucleolus oocytes.
Spawning	Highly vascularised, dark brown in colour, occupy most of the ventral cavity, large and lobular in appearance, eggs excruted with slight abdominal pressure	Predominance of germinal vesicle migratory oocyte and vitellogenic oocytes.

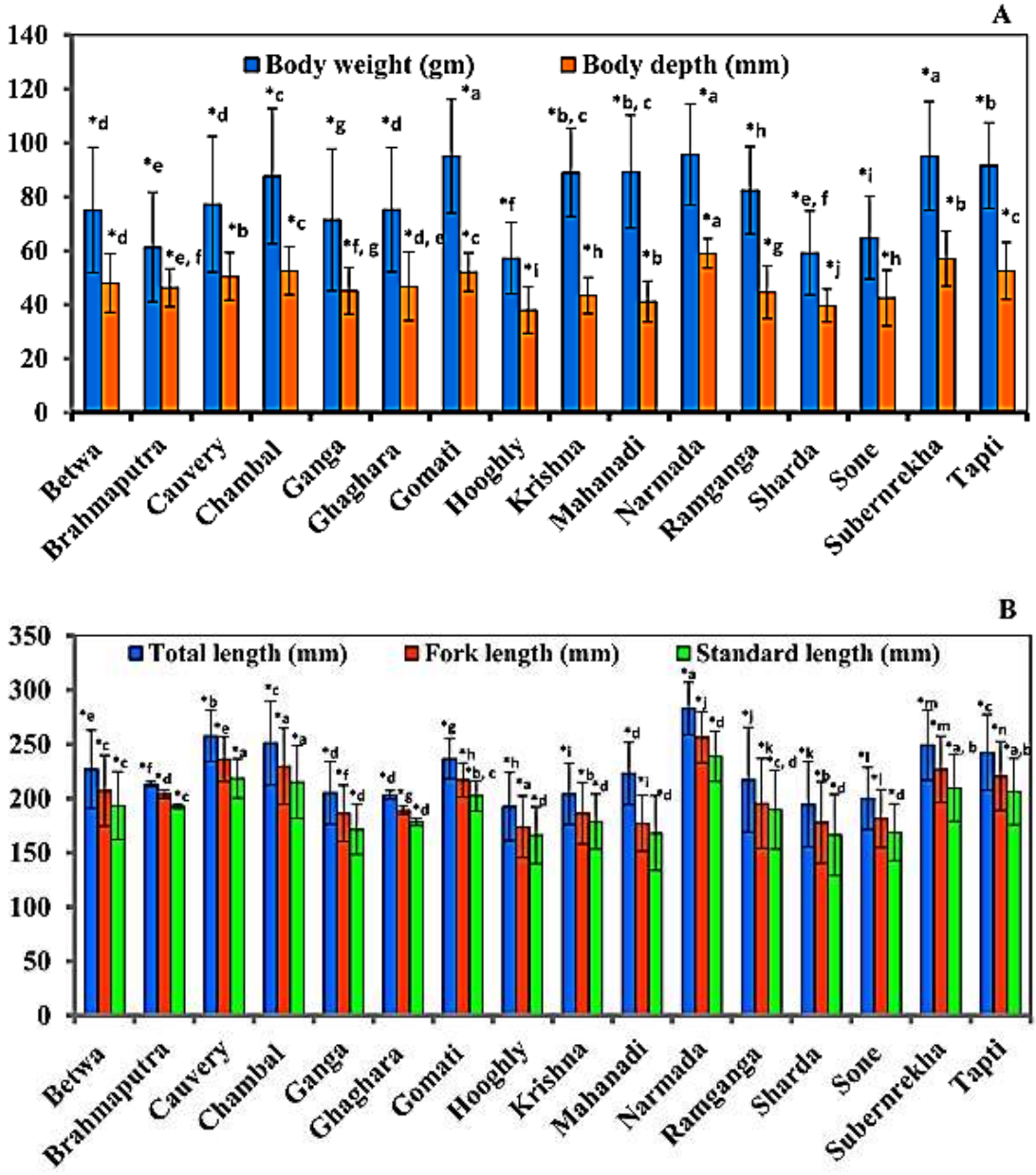

Figure 3: Showing the variations in morpho-metric parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different Indian rivers. (A): Body weight (gm) and body depth (mm) and (B): Total length (mm), Fork length (mm) and Standard length (mm). Data were expressed in mean±SEM. Asterisk showed significance at $p < 0.001$ (one way ANOVA). The bars superscripted with same letter showed no significant data (Newman-Keuls test, $p < 0.05$) whereas different letters showed significant difference among Indian rivers.

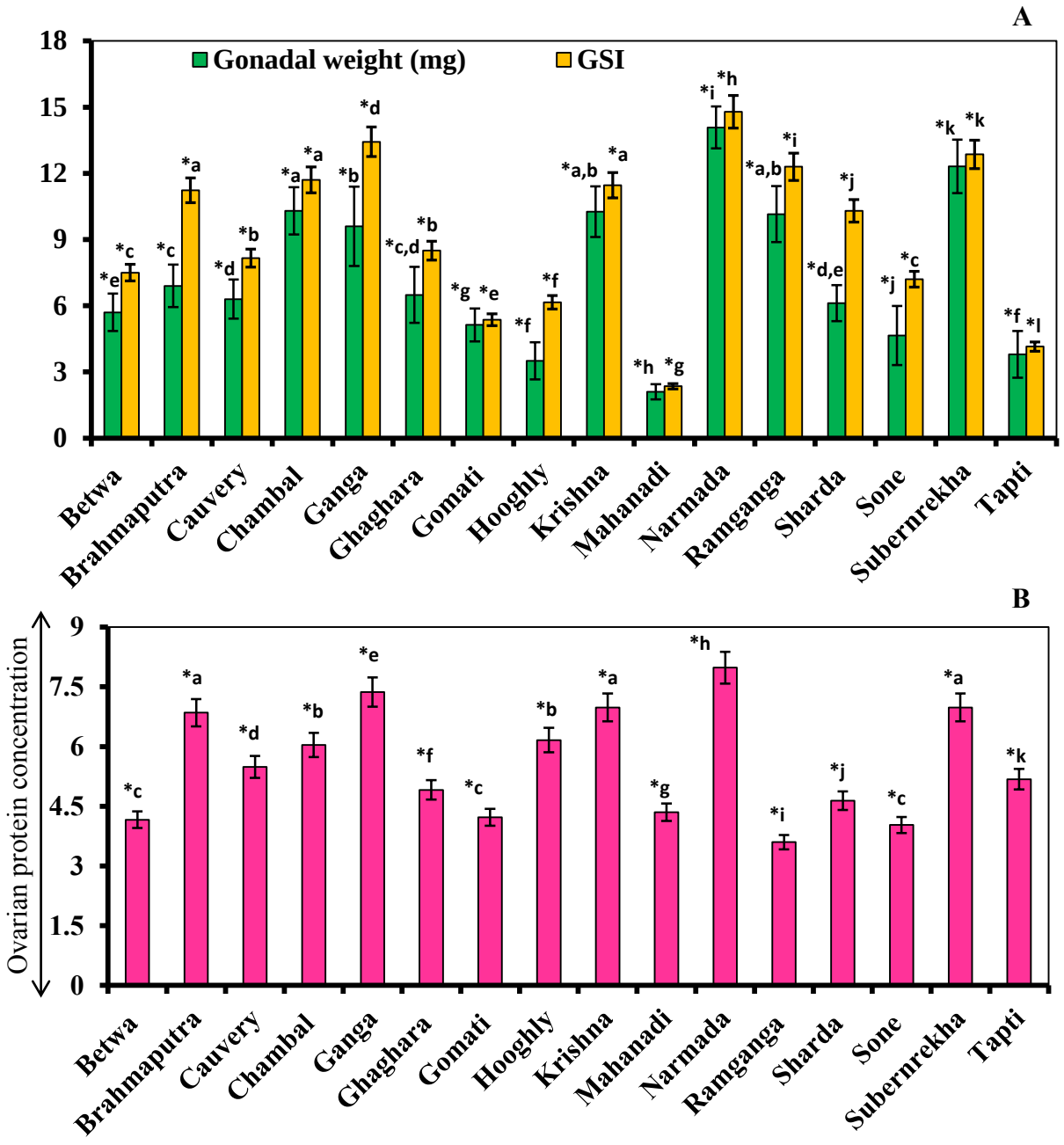

Figure 4: Showing the variations in reproductive parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) collected from different Indian rivers in relation to gonadal weight (mg) and gonadosomatic index (A), and ovarian protein concentration *(*mg/ml/100mg tissue weight) (B). Data were expressed in mean±SEM. Asterisk showed significance at $p < 0.001$ (one way ANOVA). The bars superscripted with same letter showed no significant data (Newman-Keuls test, $p < 0.05$) whereas different letters showed significant difference among Indian rivers.

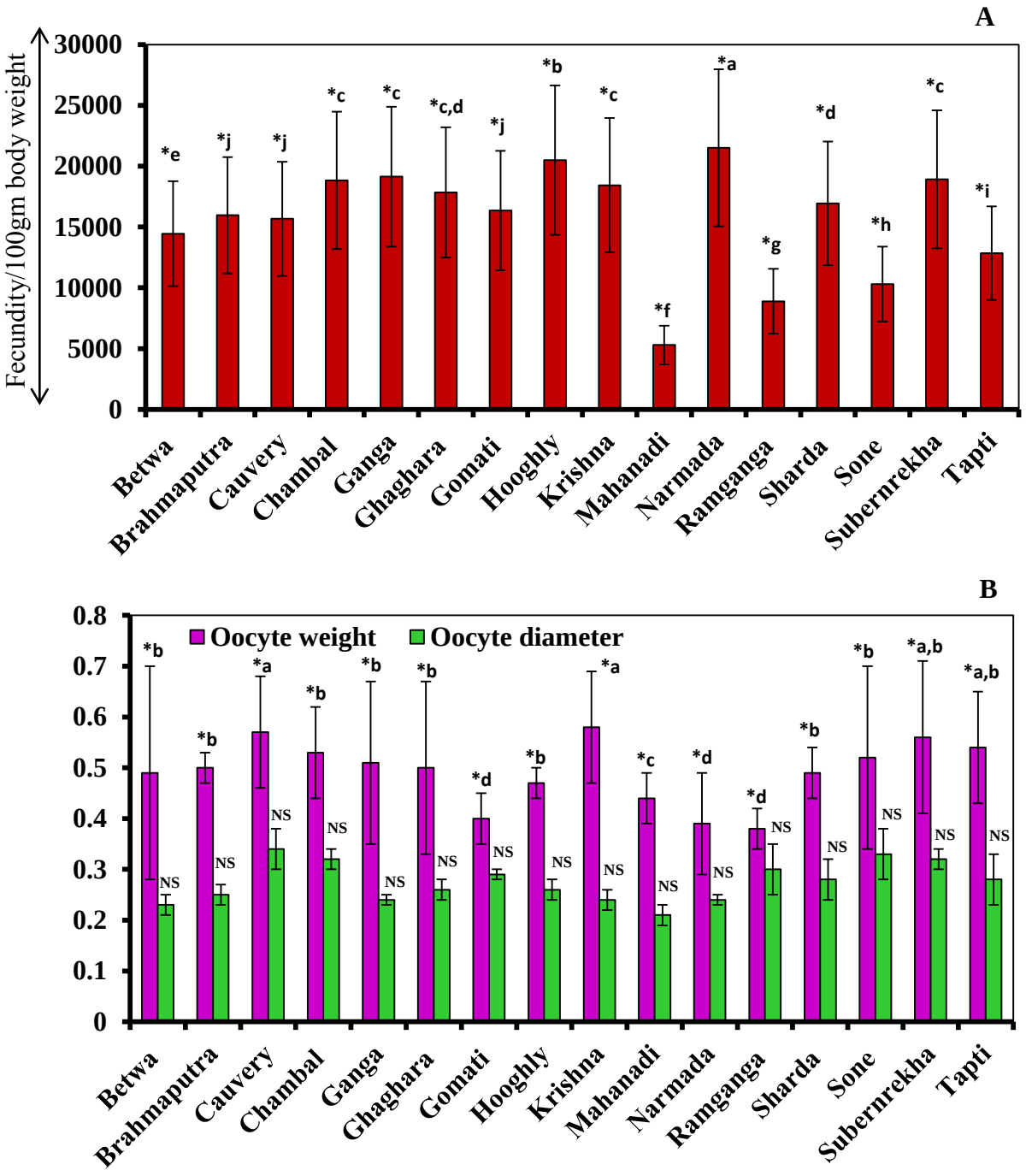

Figure 5: Showing the variations in reproductive parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) of different Indian rivers in relation to fecundity/100gmBW (A), oocyte weight (mg) and oocyte diameter (mm) (B). Data were expressed in mean±SEM. Asterisk showed significance at $p < 0.001$ (one way ANOVA). The bars superscripted with same letter showed no significant data (Newman-Keuls test, $p < 0.05$) whereas different letters showed significant difference among Indian rivers.

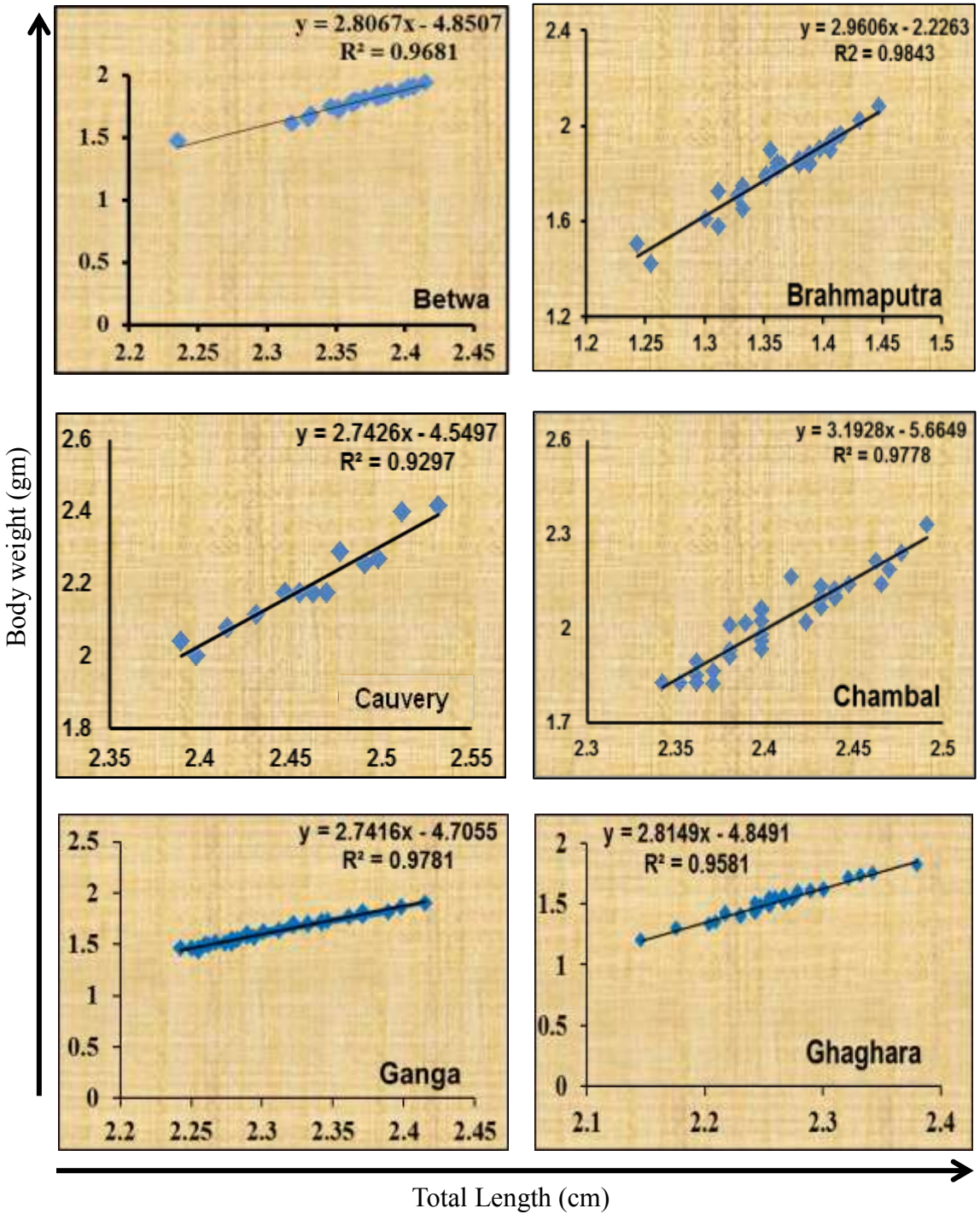

Figure 6: Regression Curve Showing the relationship between length (cm) and weight (gm) of freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794) sampled from different Indian rivers (Betwa, Brahmaputra, Cauvery, Chambal, Ganga and Ghaghara).

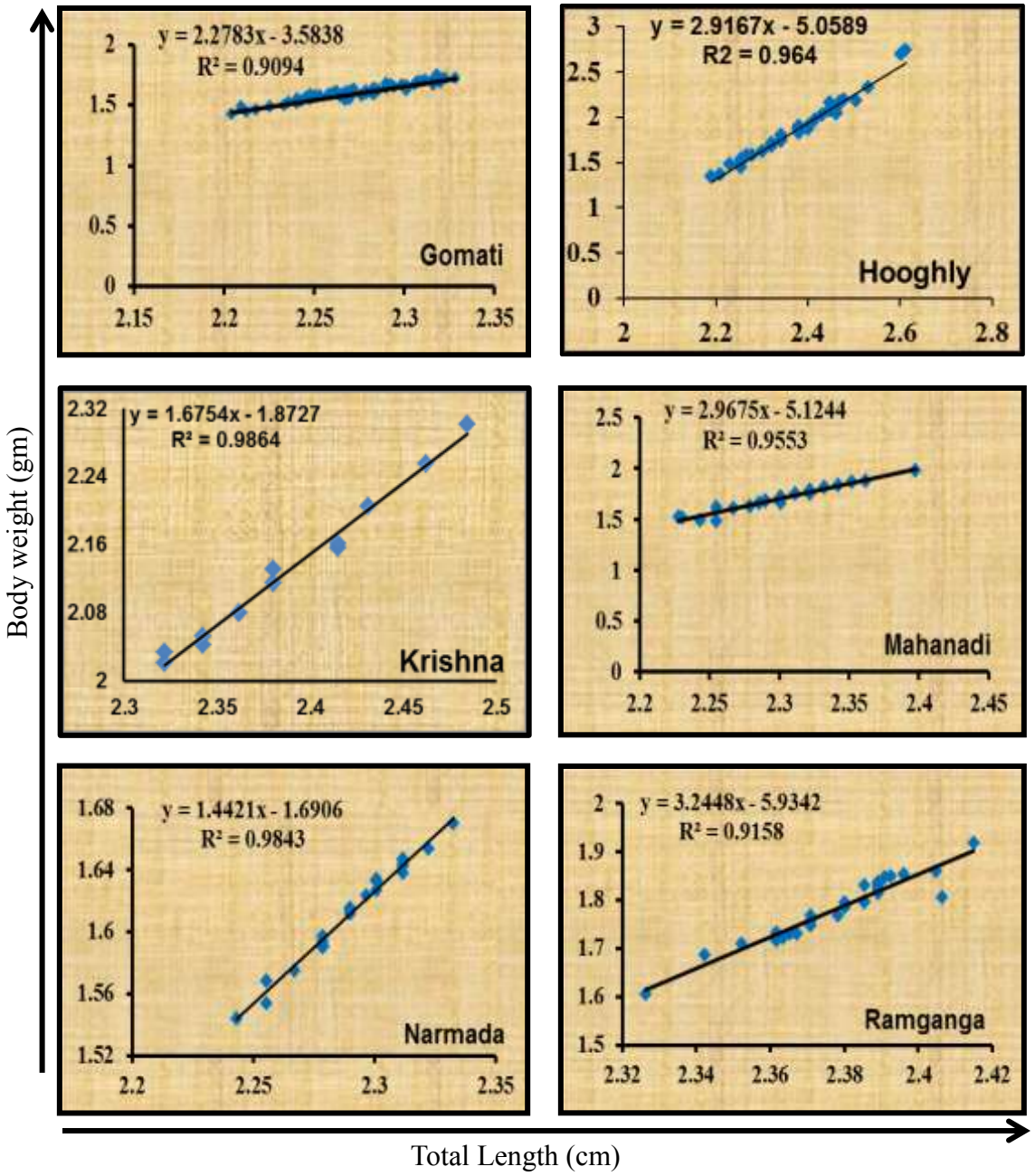

Figure 7: Regression Curve Showing the relationship between length (cm) and weight (gm) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different Indian rivers (Gomati, Hooghly, Krishna, Mahanadi, Narmada and Ramganga).

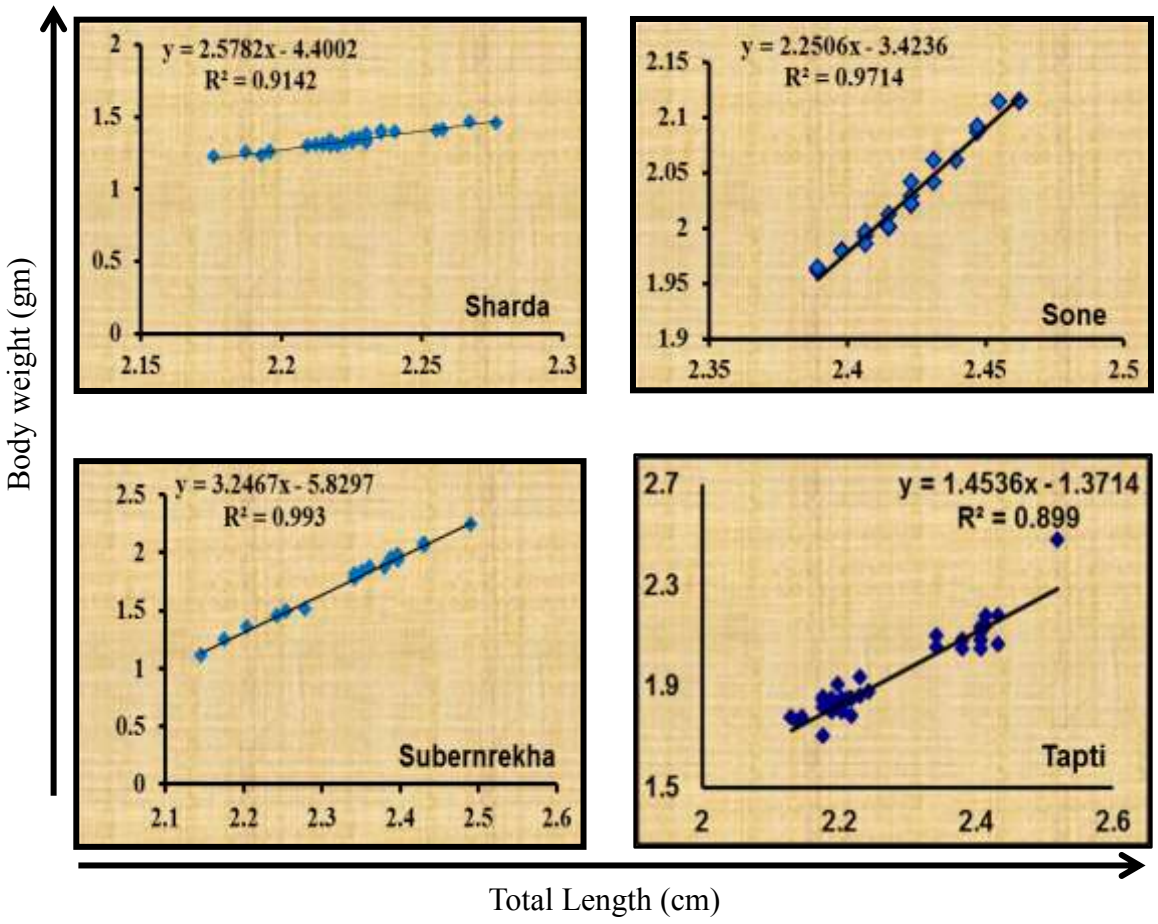

Figure 8: Regression Curve Showing the relationship between length (cm) and weight (gm) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different Indian rivers (Sharda, Sone, Subernrekha and Tapti)

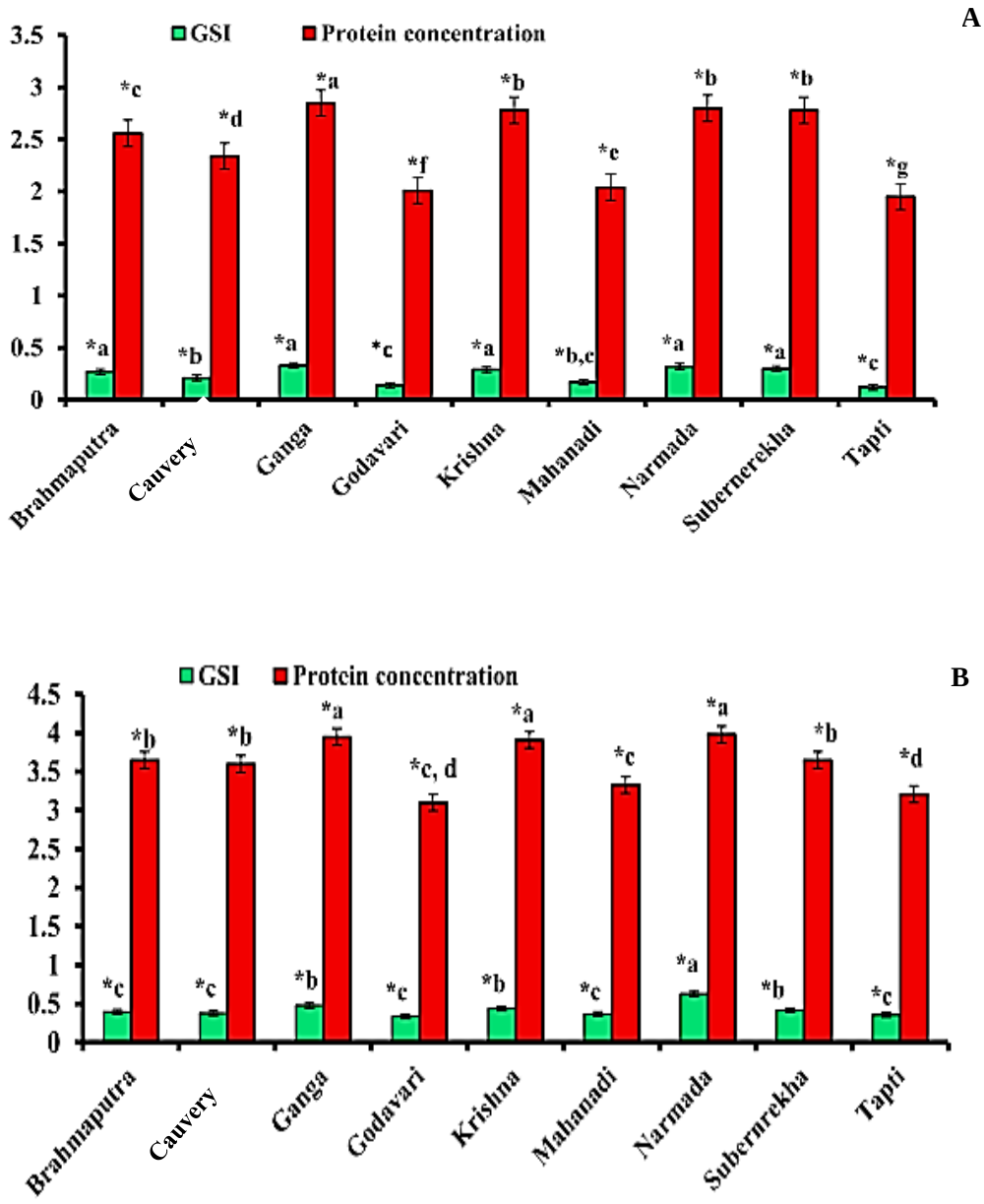

Figure 9: Showing geographical variations of freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794) of different Indian rivers in relation to gonadosomatic index (GSI) and ovarian protein concentration *(*mg/ml/100mg tissue weight) in preparatory season (A) and pre-spawning season (B). Data were expressed in mean±SEM. Asterisk showed significant at $p < 0.001$ (one way ANOVA). The bars superscripted with same letter showed no significant data (Newman-Keuls test, $p < 0.05$) whereas different letter showed significant difference among Indian rivers.

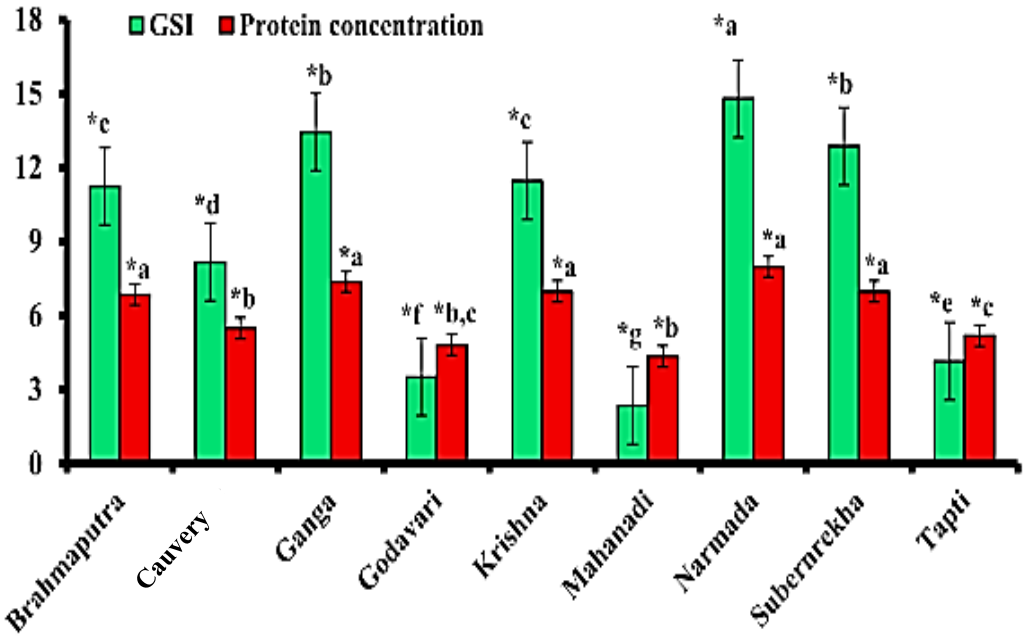

Figure 10: Showing geographical variations of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) of different Indian rivers in relation to gonadosomatic index (GSI) and ovarian protein concentration *(*mg/ml/100mg tissue weight) in spawning phase. Data were expressed in mean±SEM. Asterisk showed significant at $p < 0.001$ (one way ANOVA). The bars superscripted with same letter showed no significant data (Newman-Keuls test, $p < 0.05$) whereas different letters showed significant difference among Indian rivers.

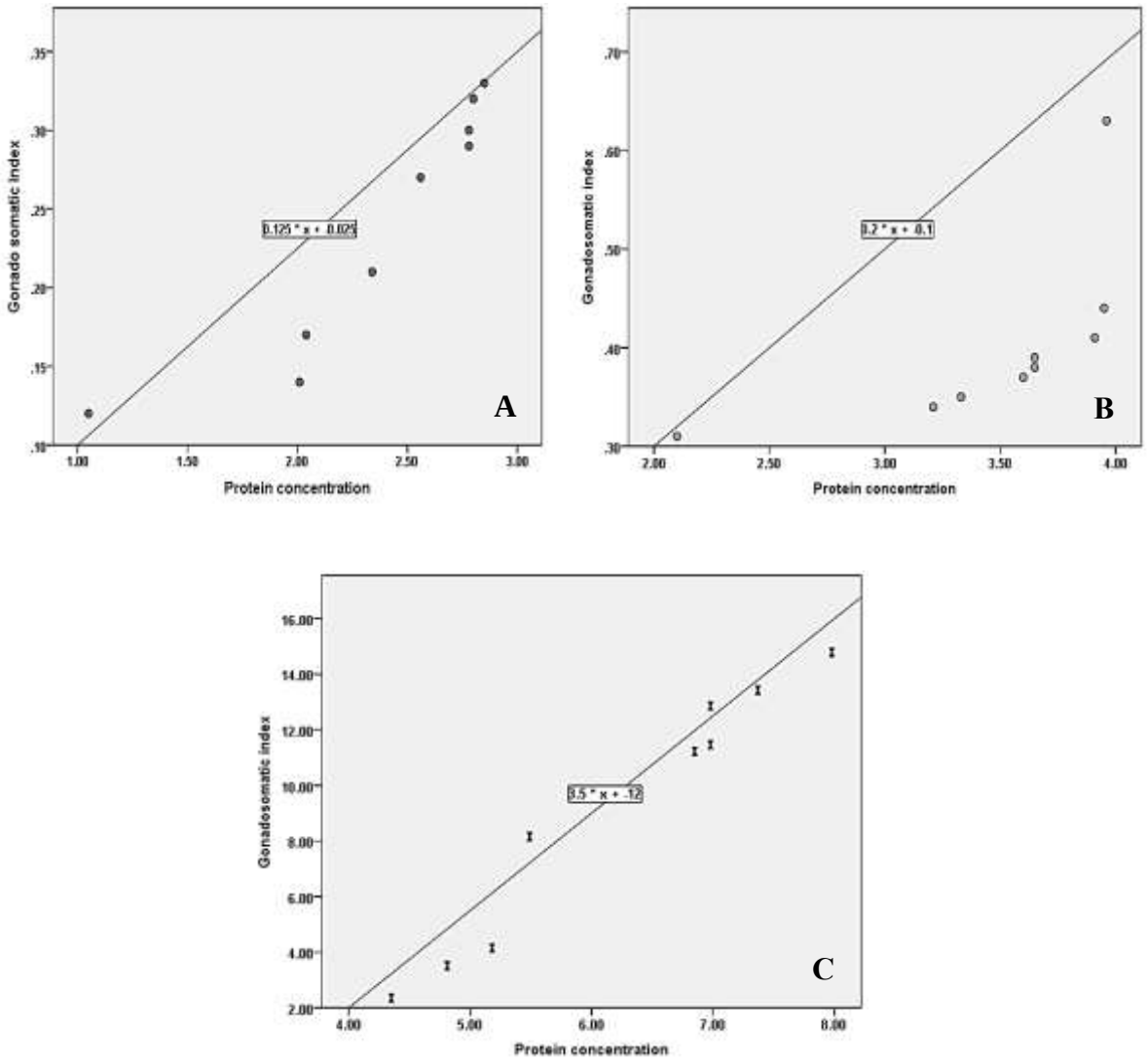

Figure 11: Showing correlations between gonadosomatic index and ovarian protein concentration of freshwater catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different Indian rivers in three phases of reproduction: A- preparatory phase, B- pre-spawning phase, C- spawning phase.

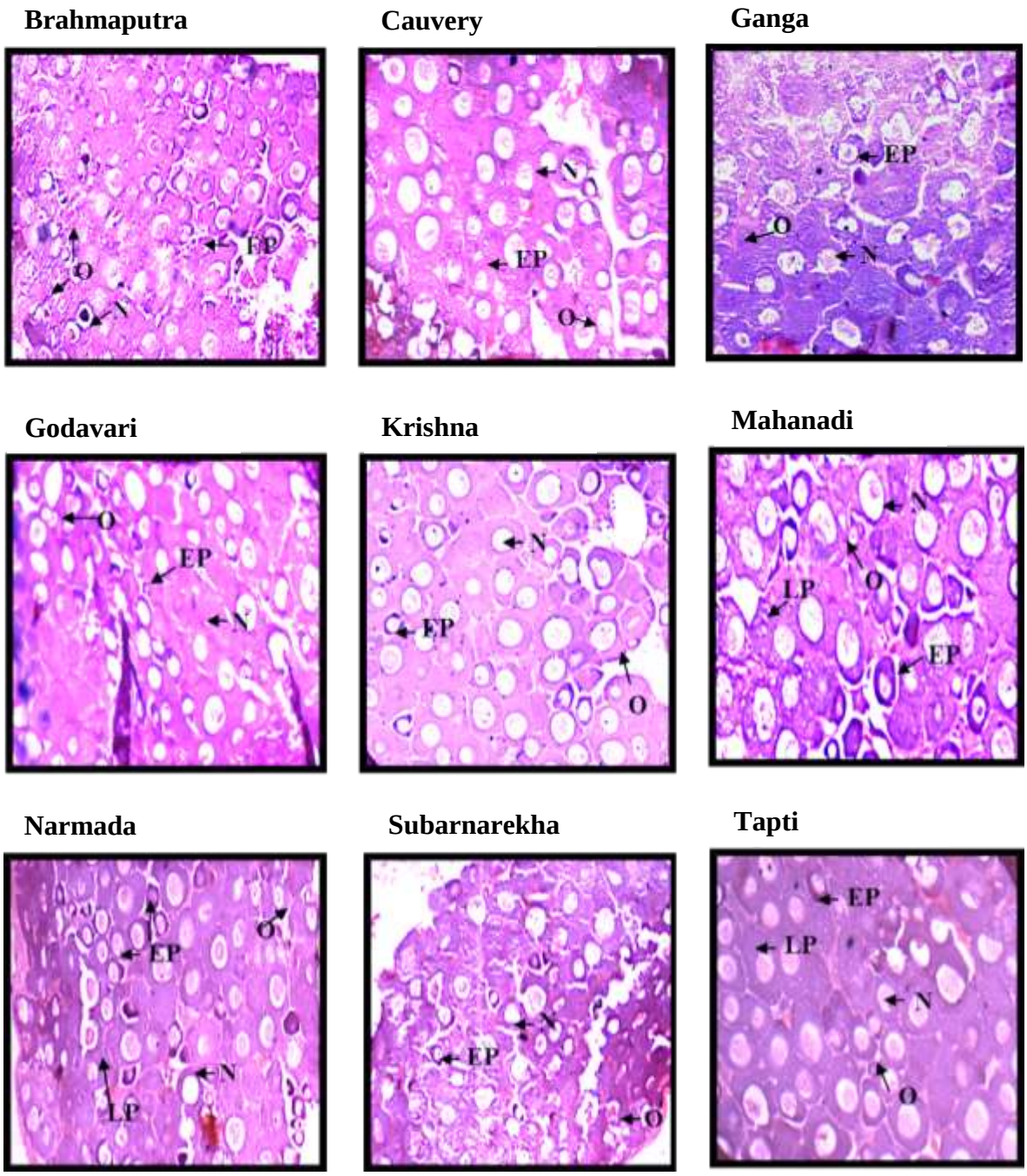

Figure 12: Histological presentation of ovarian tissue of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in preparatory phase of different explored Indian rivers viz., Brahmaputra, Cauvery, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subernrekha and Tapti. Images were acquired at 20X magnification. Note: EP- early peri-nucleolus, N- nucleus, O- oogonia and LP- late peri-nucleolus.

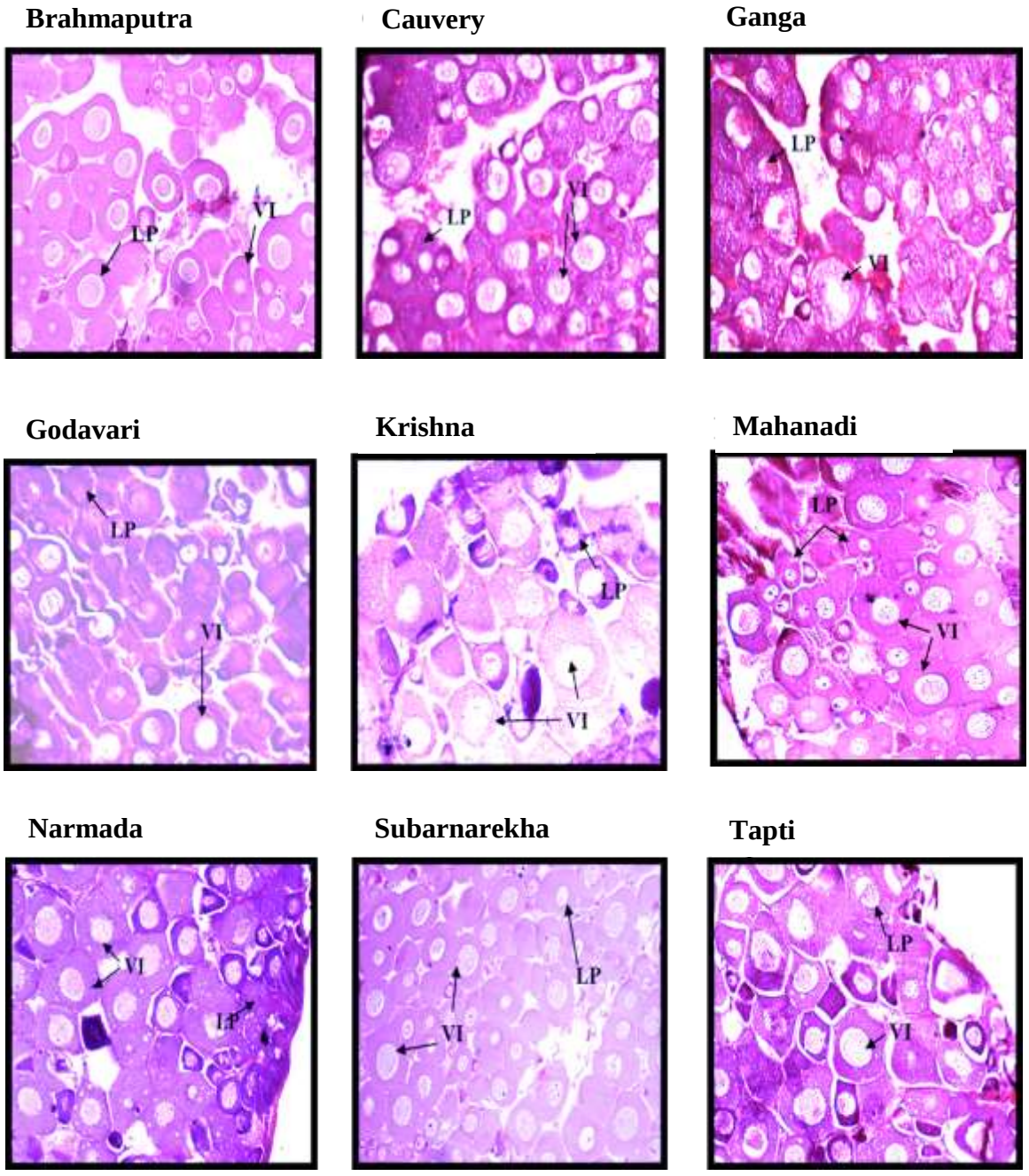

Figure 13: Histological presentation of ovarian tissue of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in pre-spawning phase of different explored Indian rivers viz., Brahmaputra, Cauvery, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subernrekha and Tapti. Images were acquired at 20X magnification. Note: LP- Late peri-nucleolus and VI: Vitellogenic oocyte.

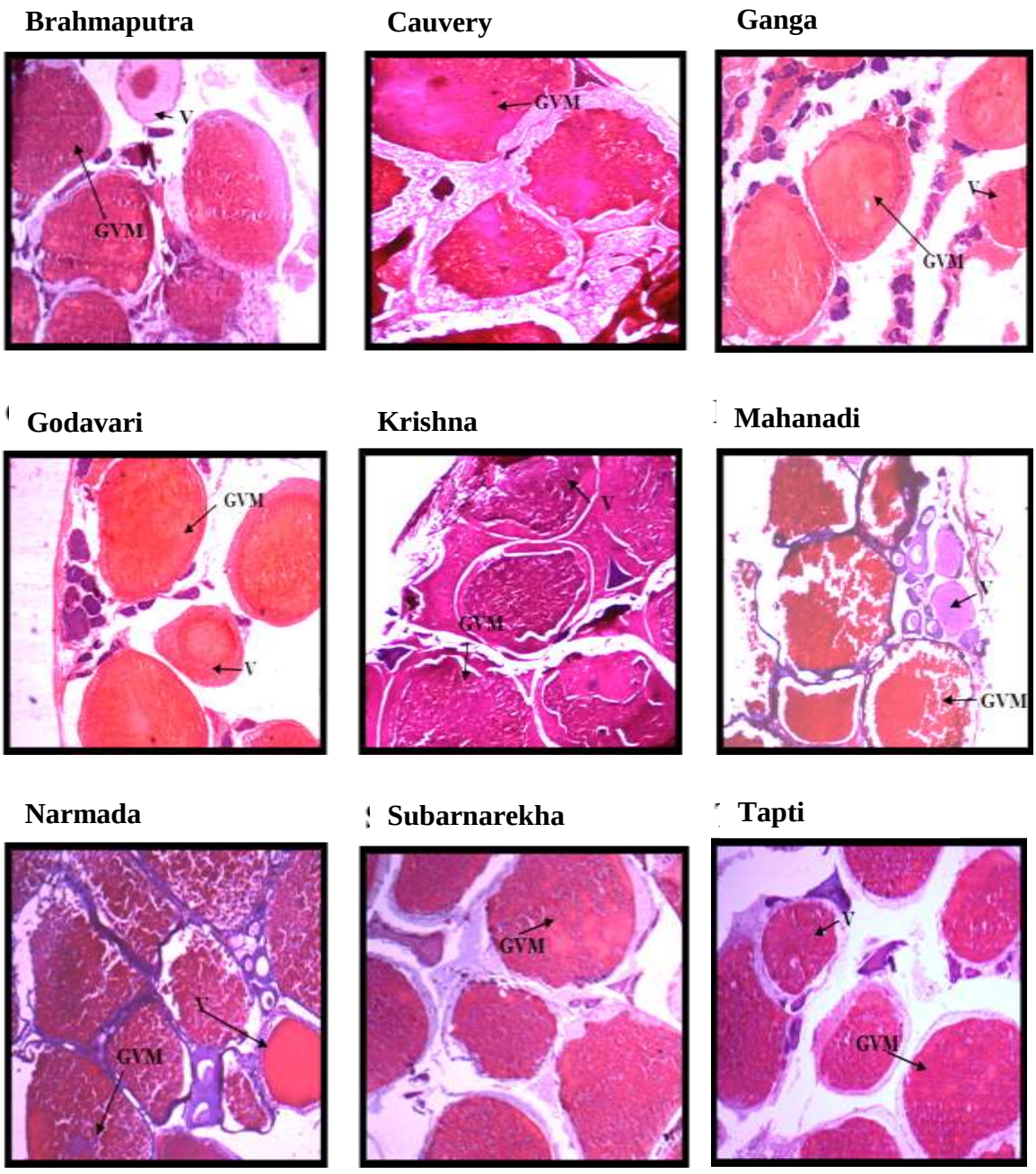

Figure 14: Histological presentation of ovarian tissue of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in spawning phase of different explored Indian rivers viz., Brahmaputra, Cauvery, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subernrekha and Tapti. Images were acquired at 20X magnification. Note: VI: Vitellogenic oocyte, GVM: Germinal vesicle migratory oocytes.

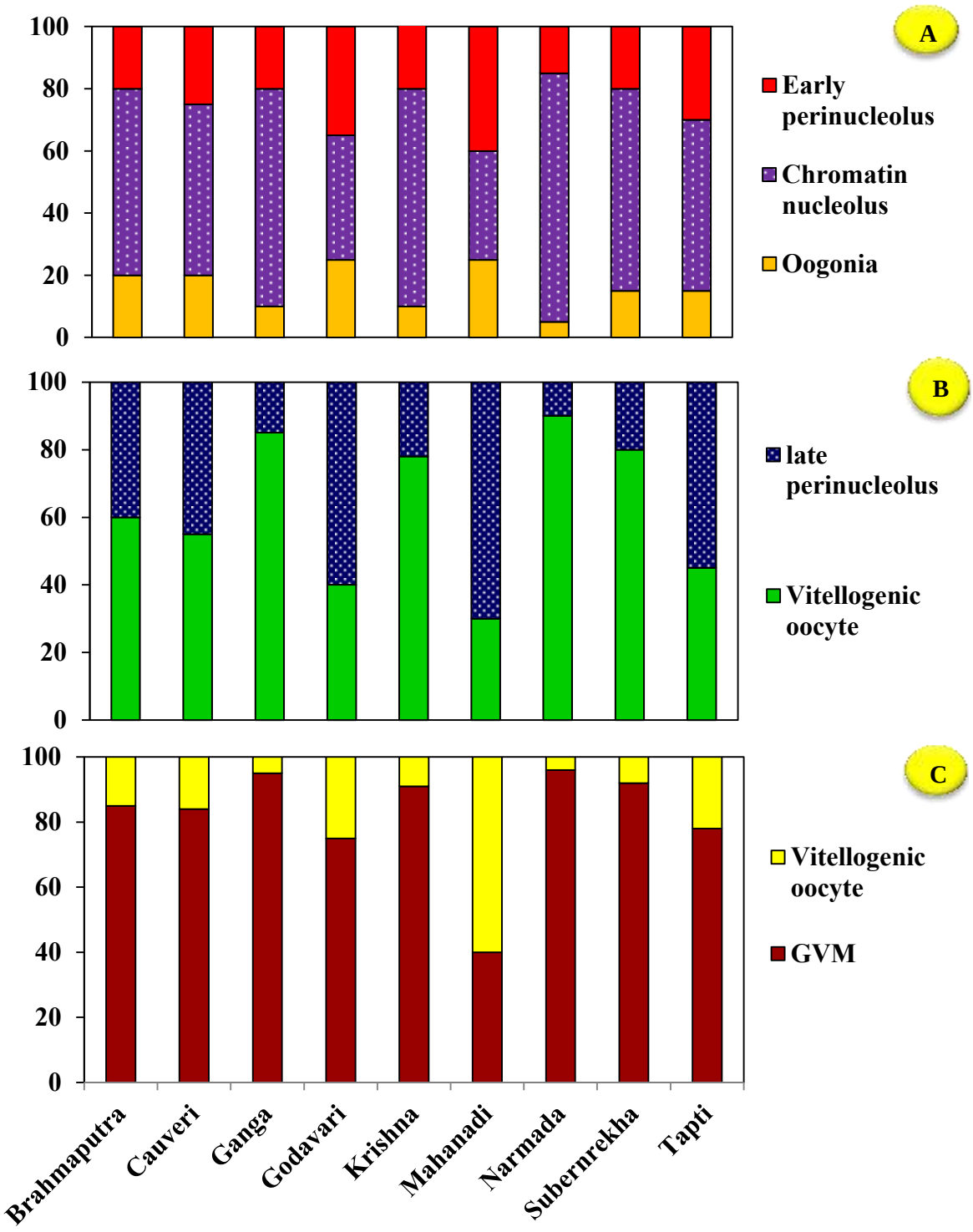

Figure 15: Showing percentage of oocyte stage frequency in different reproductive stages of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in different Indian major rivers in preparatory phase (A), pre-spawning phase (B) and spawning phase (C). Note: GVM: Germinal vesicle migratory oocyte.

4. Discussion

During the present study, five morpho-metric traits and six reproductive traits were investigated among freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from wild population of different explored Indian rivers viz., Betwa, Brahmaputra, Cauvery, Chambal, Ganga, Ghaghara, Gomati, Hooghly, Krishna, Mahanadi, Narmada, Ramganga, Sharda, Sone, Subernrekha and Tapti River. The samples of different sites were compared. Results supported significant differences between morpho-metric and reproductive parameters, fecundity, as well as in the oocyte development and size with respect to different riverine system and reproductive phases.

This analysis of fish biology of *O. bimaculatus* assists in elucidating that different aquatic habitats and environmental conditions influence morpho-metric growth and reproductive performance of fish as it affect the maturity of fish. Due to differences in geographical conditions of water bodies and environmental condition, this fish showed difference in their growth and breeding performance too. The reproductive parameters observed in this study can be attributed to the differences in abiotic factors such as photoperiod, temperature and rainfall as well as biotic factors like food availability and physiological characteristics (Azevedo, 2000) which are generally considered as determining factors that trigger the reproductive cycle (Menezes and Vazzoler, 1992).

It was observed on the basis of variations in studied parameters of *O. bimaculatus* from 16 rivers, fish can be divided in high absolute, moderate absolute and low absolute category. Though result of different parameters from varied geographical rivers, overlap with each other. One of the major keys in fish biology are morpho-metric characters as they are important for systematics, estimation of growth variability, ontogeny (Kovac and Copp, 1999) and population-level studies (Verep et al., 2006). Results showed that the healthier and longer freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) population was observed in Narmada River at Hosangabad site and small size population of *O. bimaculatus* were found in Hooghly River at Naihati site. Heterogeneity in these characteristics may be due to the difference in their habitats. Sfakianakis et al. (2011) and

Georgakopoulou et al. (2007) had concluded that morpho-metric characters were changed with water temperature. Jennings et al. (2001), King (1995) and Bagenal (1978a) reported that variations occurred in morpho-metric characteristics among similar species of fish from different geographic location to be influenced by the environmental variables.

Length-weight relationship serves as an indicator of various morphological and physiological processes (Bagenal and Tesch, 1978). This relationship was also studied by many researchers (Yusaf et al., 2009; Naeem et al., 2010a,b, 2011a,b). Fish are said to exhibit isometric growth when length increases in equal proportions with body weight for constant specific gravity. When the specific gravity of a fish remains unchanged and retains the same shape during its lifetime. The length-weight exponent (b) value fall approximately around 3.0 and can be said as isometric growth (Siegfried, 1980). The River Betwa, Ganga and Mahanadi showed isometric growth. The estimated value of b in these rivers was 2.99, 2.97, and 2.92 ($p < 0.05$). Spencer (1864-1867) described the growth of an organism through his 'cube law' which stated that 'In similarly-shaped bodies, the masses and, therefore, the weights, vary as the cubes of the dimensions, i.e., $W \alpha L^3$'. Accordingly, fish doubled its length by increasing eight times in weight (Froese, 2006). The experimentally determined value of "b" in several fish species had been shown to fluctuate around 3.0 (Ambily and Nandan, 2010; Renjini and Nandan, 2011). The value of "b" lies between 2.5 and 3.5, usually close to 3.0 (Pauly, 1984). When b=3, the growth is isometric in which it proceeds in the same dimension as the cube of length and when $b \neq 3$, the growth is allometric in that it proceeds in different dimensions (differing from L^3). Allometric growth can be either positive ($b > 3$) or negative ($b < 3$). The positive allometric growth for *O. bimaculatus* was exhibited for Hooghly, Krishna, Ramganga and Subernrekha River (b=3.28, 3.29, 3.55, 3.25; $p < 0.05$; Table 5). However, "b" value for studied relationship was lower than 3.0 or 2.9, showed negative allometric growth (Thomas et al., 2003; Pervin and Mortuza, 2008). That was represented by other explored Rivers (Brahmaputra, Cauvery, Chambal, Ghaghara, Gomati, Narmada, Sharda, Sone and Tapti River) (Table 5). When the *b* value of different wild populations was compared, River Betwa showed relatively higher b value (2.99), than River Mahanadi (2.97) and River Ganga (2.92). Tesch (1971) also reported that the

length-weight relationship in fish can be affected by habitat and area besides other factors such as seasonal effect, degree of stomach fullness, gonad maturity, sex, health, preservation technique and difference in the observed length ranges of the specimens.

Gonadosomatic index was the major determinant of reproductive development. However its use in examination of reproductive biology is more suitable when it associated with the other markers of reproduction viz., ovarian protein, fecundity, macroscopic and histological observations. The highest gonadal weight, GSI and ovarian protein was found in the sample of Narmada River (14.08±0.95 mg GW, 14.79±0.004 GSI and 7.98±0.0003 mg/ml/100mg tissue weight ovarian protein concentration) sample of *O. bimaculatus.* The increase in GSI showed advanced developmental stages of ovaries (Stoumbondi et al., 1993; Manna et al., 2010). During the preparatory phase in which the ovary showed reduced size, the GSI was about 0.12 to 0.33. In the pre-spawning phase, a wide GSI variation was shown (0.36 to 0.63). Such variation was probably due to the oocyte growth and development. In the ripe or spawning stage, GSI reached the highest value (2.35 to 14.79) due to increased weight of ovary which occupied most of the ventral cavity. GSI also showed higher variation in rainy season than the other seasons thus further confirming that the breeding or spawning take place during the rainy season (Hoque and Hossain, 1993; Roy and Hossain, 2006; Alam and Pathak, 2010). Most of the Indian freshwater teleosts attained maturity and breed during monsoon season (Encina and Lorencio, 1997; Shengde and Mane, 2006). This information of gonadosomatic index and measurement of mean ovary diameter in the preparatory, pre-spawning and spawning periods may help in fish management in determining of maturity and breeding period (Kumar et al., 2003).

The results showed that the ovarian protein level was varied considerably in the different rivers in different reproductive phases and had a linear correlation with fecundity, gonadal weight and GSI. The factors that improve ovarian protein concentration will improve fish fecundity as well as gonadosomatic index. Increase in protein content of muscle was attributed in increment with gonadal maturation which was the result of active feeding in pre-spawning phase (Srikar et al., 1979; Somvanshi, 1983;

Luzzana et al., 1996). During spawning phase (i.e. Late June to August) protein concentration was increased and reached maximum which was attributed to lower metabolic activity (Bano, 1977; Macay and Tunison, 1936). The building up of the gonad is always accomplished at the expense of body protein (Love, 1970). Therefore the protein cycle in fish can be synchronized with the fish maturity than feeding (Shreni and Kalpana, 1980).

Fecundity of studied fish was different depending on explored Indian rivers. According to Murua et al. (2003), fecundity was influenced by variation in environmental conditions such as temperature, food availability, habitat and predation intensity. It varied with the variation in reproductive characteristics of species viz., size, weight, gonadal weight and locality (Mekkawy and Hassan, 2011). The assessment of fecundity is one of the important components of reproductive biology (Nikolskii, 1963; Khallaf and Authman, 1991; Menezes and Vazoller 1992; Chech and Moyle 2004). Fecundity is a life-history trait that can be estimated by the number of oocytes that complete their development and are released in each reproductive period, i.e., reproductive investment. The findings of the present study for fecundity and oocyte diameter were parallel with the findings of Vazzoler (1996). According to Nikolskii (1969), the variation in fecundity of species that occupied different locations can be related to food supply, size of first gonadal maturation, longevity, population density, temperature and latitude. Besides, there are other factors that can interfere with fecundity, for example, the type of fertilization and care for the offspring. The findings of present investigation showed a highest fecundity in Narmada River (21512.57±5606.06) and also found a linear positive relationship between fecundity and body weight and gonadal weight of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from different Indian rivers. The findings of Sarkar et al. (2002) were in agreement with the present findings who also reported an increase in fecundity with increase in size, weight and gonadal weight in *Mystus gulio*. Fecundity exhibited adaptive fluctuations with different habitats which reflect changes in the environment. These variations may be due to food supply, temperature and habitat (Niklosky, 1969; Mekkawy and Hassan, 2011).

The correlation of coefficient and regression equations were shown in Table 3 and 4, and proved that fecundity had positive highest correlation with ovarian protein concentration, gonadal weight and gonadosomatic index. Similar results were shown by many workers viz., Khan et al. (2002) reported that a linear relationship existed between fecundity and total length, body weight and ovarian weight in *Plotosus canius*. Saifullah et al. (2004) also observed that fecundity was significantly correlated with the body length, body weight and gonad weight in *Hilsha ilisha* from Bay of Bengal. Other same reports were observed by Rao and Krishnan (2009) in *Epinephelus diacanthus* and Abedi et al. (2011) in *Garra rufa*, and Kohinoor et al. (2012) in *Notopterus chitala*. Mishra and Saksena (2012) have reported that the fecundity of *Labeo calbasu* was increasing with the increase in the fish length, fish weight and gonad weight. The fecundity provides useful information to conserve the threatened species (Hossain et al., 2012). Oocyte weight and diameter may be used as a predictor of developmental stage (Gomes et al., 2011). Both have shown significant least relationship with fecundity, ovarian protein concentration and morpho-metric parameters in all collected samples. Oocyte diameter may be used on its own to measure development, but gives little information on the physiological status of the ovaries as it was shown no correlation with ovarian protein (El-Sayed et al., 2003).

The fluctuations in gonadosomatic index, ovarian protein level and fecundity of freshwater butter catfish, *Ompok bimaculatus* associated with ovarian histology in preparatory, pre-spawning and spawning season of different Indian major rivers was also investigated. The result of histological analysis found that during preparatory season, the ovaries of *O. bimaculatus* were predominated by oocytes of peri-nuclear stage with large nuclei and many nucleoli of various sizes (Lehri, 1968, Khanna and Pant, 1996). During pre-spawning phase, ovaries were enlarged and various cytological changes were observed in the oocytes indicating rapid growth and maturation. The growth during this phase was mainly due to formation of yolk vesicles and deposition of yolk. At this stage oocytes proliferate and all types of oocytes were visible except the matured ones. In the spawning phase, GSI of *O. bimaculatus* attained a maximum peak (14.79±0.004). The ovaries during spawning phase were filled with yolk laden oocytes. Similar findings were

in many other teleostean fish (Lehri, 1968; Kumari, 2014). The histological analysis of oocytes in different developmental stages were also similar to those of other teleosts reported in the literature (Coward and Bromage, 1998; Cek et al., 2001; Utoh et al., 2003; Nejedli et al., 2004; Molen and Matallanas, 2004; Abascal and Medina, 2005; Knight et al., 2007; Koc et al., 2008; Chelemal et al., 2009; Chellappa et al., 2010; Mohamed, 2010; Morris et al., 2011).

To sum up, the obtained results of exploration of morpho-metric and reproductive parameters of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) from 16 different Indian rivers, fish smapled from Narmada River, a central Indian major river and the fifth longest river in the Indian subcontinent, had shown best sample of growth and reproductive status. So this study can suggest an idea of the best strain of *O. bimaculatus* of best sites among other rivers in favor of successful selective captive breeding. Therefore it can be concluded that the aquatic ecosystem and local environment plays an important role in the species identification, characterization and its reproductive growth.

Chapter II

Annual reproductive cycle of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in Lucknow region

ABSTRACT

The aim of the present study was to examine the annual changes in gonadosomatic index (GSI), gonadal macro- and micro-scopic changes and oocyte diameter (OD) of freshwater butter catfish, *Ompok bimaculatus* sampled from Gomati River. The obtained result showed that GSI was increased with the progressively gonadal maturation. It was increased significantly in July (4.32±0.047 in female and 0.83±0.017 in male) and it reached its peak point in the month of August (5.37±0.037 in female and 0.98±0.06 in male) and lowest value was recorded in October month (0.64±0.019 in female) and November month (0.11±0.004 in male) ($p < 0.001$). The GSI data also get support from macro-scopic monthly detail of gonads. The GSI was significantly correlated with the physico-chemical characteristics of Gomati River water. The OD was varied from 0.052±0.005 to 0.84±0.024. The maximum OD was observed in August month and lowest in September month. It showed a significant relationship with GSI. Histological studies of gonads (ovary and testis) had given a clear picture of annual reproductive cycle of freshwater butter catfish, *Ompok bimaculatus* as; resting phase (November-January month), preparatory phase (February-March month), pre-spawning phase (April-July month), spawning phase (August month) and post-spawning phase

(September-October month). The histological studies of ovary of *O. bimaculatus* reported an eight stages of oocyte development (oogonia, chromatin nucleolus, early perinucleolus, late perinucleolus, yolk vesicle, vitellogenesis, vitellogenic and post ovulatory follicles), and five types of spermatogenic cells in testis viz, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa in the seminiferous tubules during different reproductive phases. These observations are important for a better understanding of reproductive biology of this fish in northern region to adopt breeding practices of freshwater butter catfish, *Ompok bimaculatus*.

Keywords: Ompok bimaculatus, gonadosomatic index, Gonadal histology, Oocyte diameter.

1. Introduction

Ompok bimaculatus is bisexual. Sexual dimorphism is generally observed with the approach of the breeding season. Descriptions of reproductive strategies are the fundamental topics in the study of biology and population dynamics of fish species (Hunter et al., 1992). Because of its insufficiency of gravid stock, and shortage of information regarding its breeding potential, larval rearing and culture technology, this species did not get sufficient attention (Parameswaran et al., 1970; CAMP 1998; Banik et al., 2002; Banik and Malla, 2009; Malla and Banik, 2010; Banik et al., 2011). Lack of definite information on reproductive cycle and behavior of this near threatened fish species of the North riverine system is major hurdle towards its population which in turn hampered the planning and implementation of its conservation and management strategy. Studies of fish reproductive biology provide the useful information regarding its conservation programs to maintain and manage fish stocks (Chen et al., 2010, Shabanipour and Hossayni, 2010). This study helps in planning the successful breeding practices. Studies on the fish reproductive biology is essential in maintenance of healthy population, optimization of appropriate brood stock management strategies, successful fish culture and conservation programme. The reproductive biology of many freshwater species were studied by various workers, *O. bimaculatus* (Rao and Karamchandani,

1986); *Macrognathus pancalus* (Suresh et al., 2006); *Catla catla* (Deepak et al., 2008); *Platyrhina sinensis* (Kume et al., 2008); *C. chitala* (Sarkar et al., 2008); *Galaxias maculates* (Boy et al., 2009); *Astyanax fasciatus* (De Carvalho et al., 2009); *Silurus aristotelis* (Leonardos et al., 2009).

The method of studying the spawning season is to follow the seasonal changes in gonadal weight in relation to body weight, expressed as the gonadosomatic index (Ahirrao, 2002). Gonads undergo regular seasonal cyclic changes in weight indicate the spawning season (Dadzie et al., 2000). The gonadosomatic index (GSI) is an important sign of reproductive activity for the gonadal maturation determination (Hojo et al., 2004; Mishra et al., 2016). GSI particularly help in identify the time and season of breeding as the gonads swiftly increases in size and weight just prior to spawning (Belsare, 1962; Lehri, 1968; Shashi and Akela, 1996). Macro- and micro-scopic studies of gonads are a supportive stuff to confirm gonadal cycle. Histological studies offer a scope to understand the cellular kinetics of gonad, recruitment, development and reabsorption of gonadal cells and finally in staging the maturity state of the gonads (Tomkiewicz et al., 2003; Shein et al., 2004).

The understanding of gonadal maturation will also help in predicting the annual changes of fish population (Thorpe et al., 1990; Jobling et al., 2002; Tomkiewicz et al., 2003; Shein et al., 2004). The study of testicular anatomy characteristics of fish species is important in systemic and phylogeny and also gives practical knowledge related to artificial fertilization and sperm preservation (Furbock et al., 2010). Owing to large diversity of fish fauna, data on spermatogenic ultrastructure in teleosts are scarce and restricted to few species (Magalhaes et al., 2011). Therefore, a detailed study on the biological features of near threatened species will be very important for implementing any fish conservation programme (Virjenhock et al., 1998). In teleosts, two types of spermatogenesis are observed: cystic and semi-cystic. In Cystic type, the spermatogenesis is completed within the cysts whereas in semi-cystic, spermatogenesis occurs partially outside the cysts (Mattei et al., 1993). In the experimental freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794), the spermatogenesis is cystic type. Male teleost

exhibit distinguished variations in testicular structure and gamete cell arrangement like their counterpart female members (El-Gohary, 2001). In general, paired testes present dorsally in the body cavity. The lubular testis has different compartments, within them complete cycle of gamete development takes place. The germ or spermatogenic cells (SCs) are present throughout the testis in most of catfish (Chaves-Pozo et al., 2005). The SCs after meiotic division forms spermatogonia, after mitotic division it give rise to spermatocytes, spermatids and spermatozoa. Spermatogonia are large cells with vesicular nucleus. Spermatocytes are smaller than spermatogonia. Spermatids are smaller than spermatocytes with basophilic nucleus and little cytoplasm. The spermatozoa are the smallest among all the stages of testes and are mature male gamete (Santos et al., 2001). The interstitial vascular connective tissue contains leydig cells, macrophages and mast cells (Nobrega and Quagio- Grassiotto, 2007). The level of testicular activity and spermatogenesis depends mostly on the photoperiod, temperature and rainfall as in female (Garg and Sundararaj, 1985).

The oogenesis is a dynamic process in ovaries in which the oocyte passes through three various developmental stages which include primary growth phase, secondary growth phase and maturation phase (Coward and Bromage, 1998; Cek et al., 2001). The primary growth phase is categorized by pre-vitellogenic oocyte while the secondary growth phase is categorized by vitellogenic oocyte and the maturation phase by the advanced vitellogenic oocyte undergoing germinal vesicle migration (Estay et al., 1998). Majority of teleost breed once in a year particularly in monsoon or rainy season and to perform spawning in monsoon season, maturation of gonads takes place in pre-monsoon season. Marza (1938) was first to described the synchronous and group-synchronous pattern of oocyte development. Most teleostean species showed annual breeding, follow the synchronous pattern of oocyte development which is controlled by environmental factors (De Vlaming, 1975). The reproductive cycle of teleost may be divided in to four groups viz., preparatory, pre-spawning, spawning and post-spawning phase (Sundararaj and Vasal, 1976; Chakraborty and Bhattacharya, 1984). There are a few methods to determine the gonadal development stages such as oocyte size measurement, macroscopic study of whole oocyte, external appearance of ovary, gonadal index and

histology. The histology is the most accurate method for the assessing the gonadal development (Hibiya, 1982; West, 1990). Morphological changes in developing oocytes have been described for several species of teleosts (Grau et al., 2009; Chen et al., 2010; Lubzens et al., 2010; Mohamed, 2010) including changes in gonad and oocyte size, the nucleolus (Thiry and Poncin, 2005), follicular epithelium (Quagio-Grassiotto and Guimaraes, 2003) and development of the yolk (Hartling and Kunkel,1999). The development of the oocytes may have unique characteristic features, such as GSI and the size of oocytes, which are different in different months. In aquaculture it has not received much attention due to insufficiency of gravid stock for experimentation and also because of shortage of information regarding its breeding potential, larval rearing and culture technology (CAMP, 1998; Banik et al., 2011).

There are few studies in India regarding the reproductive biology of *O. bimaculatus* for their effective management. With the exception of a preliminary information on aspects for *Ompok pabda* on breeding and seed production (Bhowmik et al., 2000; Sarkar et al., 2005), reproduction and fecundity in Bangladesh (Hossain et al., 1992), histological analysis of gonadal development (Begum, 1997) and chromosomal studies by Datta et al. (2003). Banik et al. (2012) studied the reproductive physiology of this fish species. They observed some parameters such as GSI, sex-ratio, fecundity, maturity stages, egg diameter etc. They found that maturity stage of *Ompok pabda* was developed in monsoon season during May to August month. First maturity stage was noticed during May-June and peak spawning period in June-July month. The egg attained greater diameter during June to early-August month. Siddiqua et al. (2000) conducted a study on histology of spermatogenesis of *Ompok pabda*.

The aim of the current study was to describe the gonadosomatic index and determination of the gonadal developmental stages of freshwater butter catfish, *Ompok bimaculatus* collected from Gomati River, Lucknow, U.P. The information on the annual gonadal development of freshwater butter catfish *Ompok bimaculatus,* of Gomati River is scarce. Therefore this study would provide useful information on its spawning pattern and its strategy, which is very important for its conservation and culture programme in

this state. Through this study, I tried to fill this gap with the belief that this information will be useful in the management of this near-threatened fish species especially in North India.

2. Materials and Methods

2.1. Chemicals

All the chemicals used in this analysis were of analytical grade and purchased locally from scientific suppliers, Lucknow, Uttar Pradesh, India.

2.2. Sampling sites and sample collection

The fish were handled in accordance with local/national guidelines for experimentation on animals and all care was taken to prevent cruelty of any kind.

The Gomati River, a major tributary of the Ganga River system has been selected for sample collection of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794). The river originates from a natural lake in the forested area (elevation of about 200 m; North latitude $28^{o}34'$ and East longitude 80^{o} 07') near Mainkot (around 3 km east of Pilibhit district in Uttar Pradesh, about 50 km south of the Himalayan foothills) (Singh et al., 2004). Lucknow (population about 3.4 million) is the major urban settlement on the river bank. The river serves as a major source of domestic water supply of the Lucknow city, the State capital of Uttar Pradesh, India. The present study was conducted during October 2014 to September 2015. The meteorological data of observational period of Lucknow were shown in Figure 16 which was collected from Indian Institute of Sugarcane Research, Lucknow.

The freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) were collected from different sampling zones of Gomati River, Lucknow through drag nets with the help of local fisherman (Figure 17). The sampling sites were divided in to four locations based on the similarity of the physical habitat and distance coverage of each location (total 6.5

km). These were Daliganj (location 1), Hanuman setu (location 2), Boat club (location 3) and Gomati barrage (location 4). The stream type in mentioned sampling locations was mid-stream. Fish sampling locations were documented using global positioning system. After collection, fish were brought to the laboratory in wide mouth plastic container with proper care and aeration. In the laboratory, fish were treated with 0.05% $KMnO_4$ solution to remove dermal infection if any. They were acclimatized in 200 lt glass aquaria for two weeks. Water was renewed daily to remove faecal matter and waste metabolite during acclimatization. Fish were fed with dried shrimp daily at particular time intervals.

2.3. Physico-chemical characteristics of Gomati River

The water samples of Gomati River were collected at monthly intervals during the first week of each month throughout the study period (from October 2014 to September 2015) between 8:00 AM to 10:00 AM. These were collected in five replicates. The samples were analysed for physico-chemical parameters viz., pH, temperature, dissolved oxygen (DO), free carbon dioxide (CO_2), total hardness and alkalinity according to the standard methods documented by APHA (2005).

2.3.1. pH

The hydrogen ion concentration (pH) of Gomati River water was measured with 0.1 accuracy electronic pH meter (Elico) annually.

2.3.2. Temperature

The water temperature was recorded for each month with the help of mercury thermometer and expressed in ^{o}C.

2.3.3. Dissolved oxygen (DO)

The dissolved oxygen of Gomati River was monitored by Winkler's method using manganous sulphate and alkali-iodide-azide solution (Golterman et al., 1978). For this

analysis, the water samples of each month were collected in 250ml bottles. Manganous sulphate (2 ml) and alkaline iodide azide (2 ml) solution were added to water sample to fix the dissolved oxygen. Then it was shaken rapidly and brown precipitate was occurred. The precipitation was allowed to settle. Then further, concentrated H_2SO_4 (about 2 ml) was added slowly through the wall of bottle and well shaken to dissolve the precipitate. Titrate 50 ml of this solution against 0.025 N sodium thio-sulphate solution by using indicator as starch to the colorless endpoint. The dissolved oxygen was calculated by given formula:

Dissolved oxygen (mg//l) = (XxNx8x1000)/Y

Where X= quantity of titrant used (ml)

Y= water sample (ml)

N= Normality of titrant

2.3.4. Free carbon dioxide (Free CO_2)

It was measured by phenolphthalein indicator and sodium hydroxide as a titrant. 5 drops of phenolphthalein indicator was mixed in 50 ml of water sample. If the colour of water sample was changed to pink then it was indicated the presence of free carbon dioxide in the water. Otherwise, in case of colour-less water sample, 0.22 N sodium hydroxide was added in this sample till the water colour permanently changed to pink. Free carbon dioxide was estimated as given formula:

Free CO_2 (mg/l) = (XxNx8x1000)/Y

Where X= quantity of titrant used (ml)

Y= water sample (ml)

N= Normality of titrant

2.3.5. Total hardness

Total hardness of the water was determined by EDTA titration method. To estimate the total hardness of sample, 1 ml of ammonia buffer and a pinch of erichrome black-T indicator were added to 50 ml water sample. Further, the made solution was titrate with 0.01 M EDTA till the colour of the solution was changed to blue from purple. The total hardness was calculated as:

Total hardness (mg/l) = (XxZx1000)/Y

Where X= quantity of titrant used (ml)

Y= water sample (ml)

Z= mg $CaCO_3$ equivalent to 1 ml EDTA

2.3.6. Alkalinity

To estimate alkalinity of the water sample, 2 drops of phenolphthalein indicator was added to 50 ml of water sample. Water colour was changed if phenolphthalein alkalinity was present. In the solution was colourless, 2 drops of methyl orange indicator was added. Further, the solution was titrated against 0.02 N sulphuric acid until the colour was changed to faint orange from yellow as its endpoint. The alkalinity was estimated as given formula:

Alkalinity (mg/l) = (Xx1000x0.02x50)/Y

Where X= quantity of titrant used (ml)

Y= water sample (ml)

2.4. Experimental design

2.4.1. Gonadosomatic Index

It was defined as the percentage of fish total weight represented by their gonads (Vazzoler, 1996). The fish were weighed using digital balance (Shimadzu-AY220; with

0.01 g accuracy) and sacrificed to remove gonads. Gonadal weight was recorded. GSI for every month was calculated with following formula (Lagler, 1956):

GSI= Gonadal weight x 100 / Body weight

2.4.2. Histological analysis

After the acclimatization of experimental fish, gonadal tissues (Ovary and Testis) were dissected out for histological process after sacrificing fish. They were first washed in normal saline for further process of histology. The procedure of histology was already described in chapter 1 as Table 2.

2.4.3. Oocyte diameter

To find out the oocyte diameter, the ovaries were first preserved in 5% formalin. Further, 20 ova were taken randomly from the preserved ovaries sample as three portions from each ovary (anterior, middle and posterior region). These samples were spread uniformly over a glass slide. The diameters of ova were measured in a straight line under compound microscope at 10x magnification using stage and eye-ocular micrometer. One micrometer division (m.d.) of the ocular micrometer was equal to 0.016 mm.

2.4.4. Oocyte stage counting

Different oocyte stages were counted by Image analysis software (Magnus Pro) in annual sections of ovary. The microscopic developmental stages of oocyte were categories according to Janssen et al. (1995) and the atretic oocytes and post ovulatory follicles histological classification were followed by Hunter and Macewicz (1985) and Hunter et al. (1986). Month wise percentage frequencies of different stages of ova were calculated using the formula: (number of ova of particular stage/Total ova counted) x 100.

2.5. Statistical analysis

The data were expressed at mean±SEM. The one way analysis of variance (ANOVA) was used for overall significance ($p < 0.001$) and Newman-Keuls test ($p < 0.05$) was done to get inter-group variation. Pearson correlation was performed to analyze the correlation between physico-chemical characteristics of Gomati River water, and male and female gonadosomatic index at $p < 0.05$ significance level. This correlation was done by IBM SPSS software (version 20.0). The histological sections of gonadal tissues were examined under Bright field microscope (Olympus CX41) using micropublisher 3.3 RTV camera (Qimaging, BC, Canada) at 20X magnification.

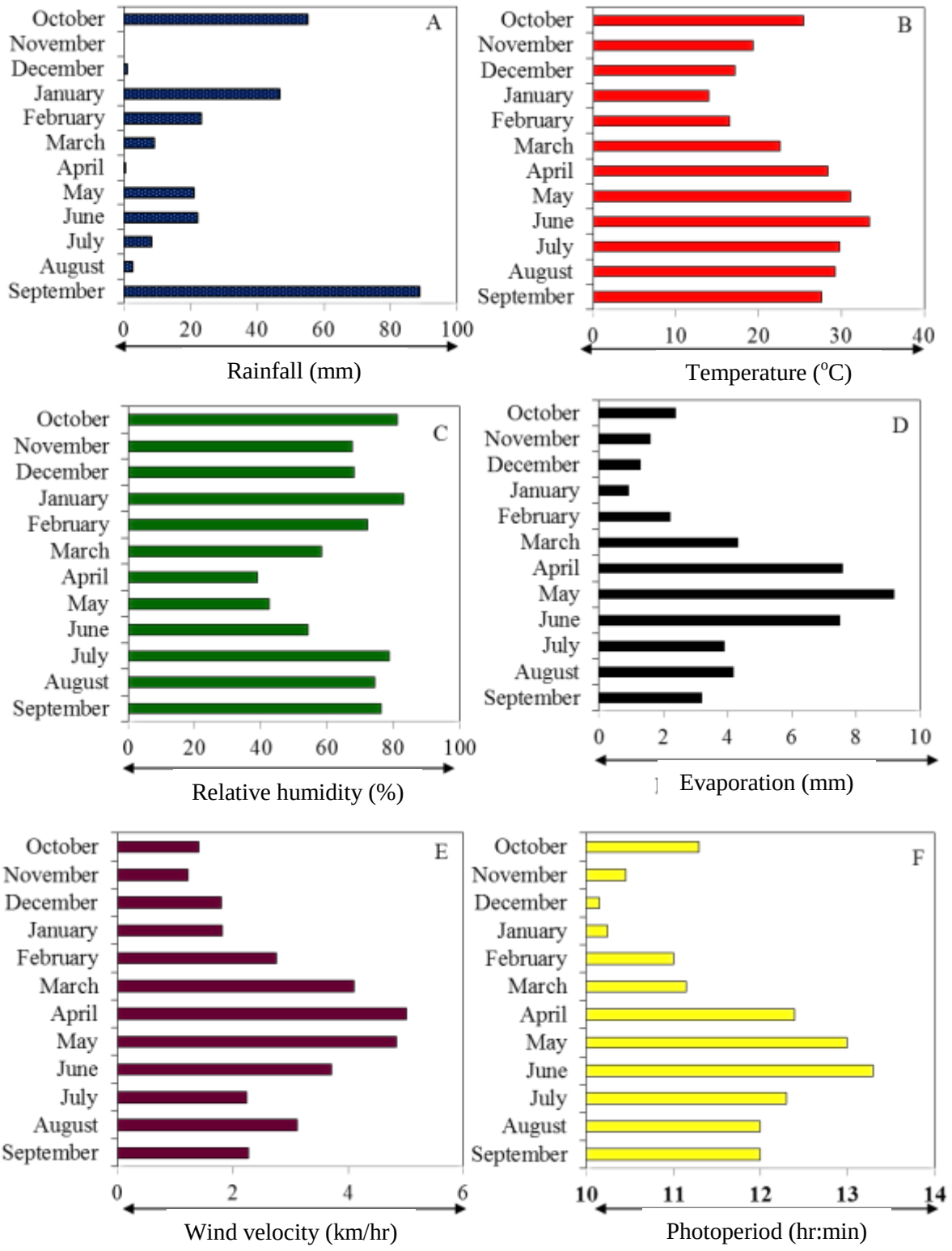

Figure 16: Showing the annual variation in meteorological data of Lucknow region, Uttar Pradesh, India observed during October 2014 to September 2015. Graph showed (A): Rainfall (mm), (B): Temperature (°C), (C): Relative humidity (%) (RH), (D): Evaporation (mm), (E): Wind velocity (km/hr) (F): Photoperiod (hr:min).

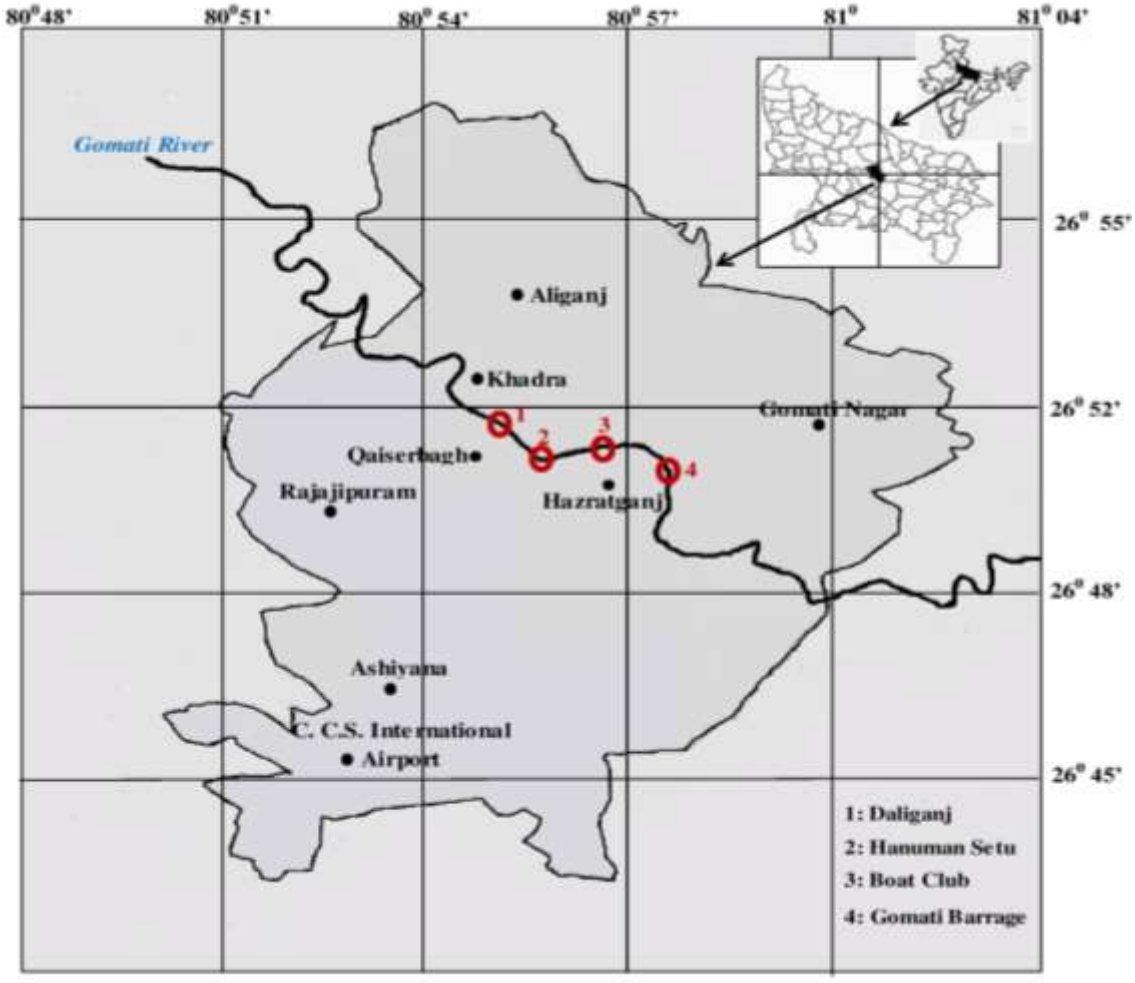

Figure 17: Drainage map of Gomati River, Lucknow, Uttar Pradesh, India showing different sampling sites for collection of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

3. Results

3.1. Physico-chemical characteristics of Gomati River water

The physico-chemical characteristics of Gomati River water provide a fair idea of water quality (Figure 18). All the physico-chemical parameters of Gomati river water were within the highest desirable limit or maximum permissible limit set by WHO (WHO, 1993). The physico-chemical characteristics of Gomati River water varied annually with different month or season were significant ($p < 0.05$). The range of water temperature was varied as expected with seasonal climates ranged from 19±0.3 ^{o}C to 30.2±0.4 ^{o}C and averaged 24.65 ^{o}C. The minimum mean water temperature was recorded in January month (19 ^{o}C) and maximum in June month (30.2 ^{o}C) respectively (Figure 18). Like temperature, water pH also varied seasonally between 7.6 to 8.33±0.2. The minimum water pH was found in October (7.6) and January month (7.6) and maximum pH in June month (8.33). There were small fluctuations in water pH from October to April month (7.6-7.99). Thereafter an increment was noticed in pH from May (8.19) to June (8.33). The spatial distribution of pH (7.2- 8.3) suggested the alkaline nature of the Gomati river water. The average seasonal concentration of dissolved oxygen (DO) was found to range between 6.3±0.1 to 10.8±0.2 mg/l. The level of DO was observed maximum in June and July month (8.97±0.2 and 10.8±0.2) whereas the lowest value was recorded in December, January and February month (6.5±0, 6.3±0.1 and 6.89±0.1). The total hardness and free CO_2 were ranged from 185±0.7 to 288±1 mg/l and 32.12±0.1 to 48.93±0 mg/l. The decrease level of free carbon dioxide was observed in the month of January (32.12±0.1 mg/l) and high value in April, May and June month (42.15±0.1, 48.93±0, 44.08±0.2 mg/l). The high value of total hardness was observed in June month. The relatively low value was measured in December month. The maximum alkalinity as $CaCO_3$ was observed in September month (273±0.6 mg/l) while minimum value was observed in January month (129±0.6 mg/l).

3.2. Correlations between various physico-chemical characteristics of Gomati River water

Pearson correlation coefficient was calculated between the water quality parameters of Gomati River. The values of correlations coefficient (r) were presented in Table 8. The result of coefficient of determination (r^2) among parameters showed that water temperature had strong positive correlation with free CO_2 ($r^2 = 0.782$). This showed that with increase in water temperature, the free CO_2 was also increased. Similarly pH showed a significant positive correlation ($r^2= 0.714$) with total hardness (TH). This expressed that pH was proportional to total hardness. It meant with increase or decrease in pH, total hardness was also increased or decreased. Temperature showed a moderate relationship with pH ($r^2= 0.579$), TH ($r^2= 0.57$), alkalinity ($r^2= 0.389$) and DO ($r^2= 0.569$). Water pH also showed moderate relationship with DO ($r^2= 0.308$) and free CO_2 ($r^2= 0.368$), and low correlation showed with alkalinity ($r^2= 0.016$). Free CO_2 exhibit a significant correlation with total hardness ($r^2= 0.41$), alkalinity ($r^2= 0.526$) and DO ($r^2=0.312$). The total hardness showed least correlation with alkalinity ($r^2= 0.006$) and DO ($r^2= 0.143$). A significant positive correlation was found between alkalinity and DO ($r^2=0.384$).

3.3. Gonadosomatic index

3.3.1. In Male Ompok bimaculatus fish

In the present study, it has been observed that the values of GSI in male freshwater butter catfish, *Ompok bimaculatus* followed a regular seasonal change from the observational period from October 2014 to September 2015 during their all reproductive phase's viz., immature, early development, maturing, spawning and post-spawning phases ($F=64.47$ at $p < 0.005$; Figure 19A). The value of GSI was ranged from 0.11±0.004 to 0.98±0.06. The mean GSI of the fish tend to increase as the fish reached maturity and after spawning, it declined and the minimum GSI was recorded during resting phase. The lowest GSI value was noticed during the end of post spawning phase

in November month (0.11±0.004). During December month i.e. immature phase, the mean GSI value increased subsequently (0.12±0.004). Further during immature phase in January and February month, GSI was gradually increased (0.12±0.0017 to 0.44±0.04). However, from March month (0.5±0.04) or onwards when the testes entered into the early development or preparatory phase, GSI was gradually increased up to July (0.83±0.017) month. GSI was raised sharply in August month (spawning phase: 0.98±0.06). Testes were constituted with full of spermatids and spermatozoa and the GSI rised up to peak value in spawning phase. A sharp decrease in GSI value was noticed in the post spawning period i.e. in month of September and October (0.36±0.03 to 0.16±0.004). GSI value was significantly declined as the testes suffered from a regression state (Figure 19A).

3.3.2. In Female Ompok bimaculatus fish

GSI of female freshwater butter catfish, *Ompok bimaculatus* fish was ranged from 0.64±0.019 to 5.37±0.037 respectively from October month to August month viz., immature to spent stage (or from resting phase to post-spawning phase) of gonadal development (Figure 19B). The highest GSI was noticed from the month of July to August (4.32±0.047 to 5.37±0.037) i.e., spawning phase and peak value of GSI was recorded in August month when ovary was attained full maturity. After this phase, the reproduction cycle undergoes post-spawning phase, at this time, GSI declined in the September (2.93±0.126) and sharply dropped in October month (0.64±0.019). There was a gradual increase in GSI after the post-spawning and resting phase (November to February month) because the ovary weight was increased gradually. The GSI was found to be minimum in November to February month (0.76±0.017 to 1.84±0.034). Further the reproductive cycle proceed in to preparatory phase from March to April month and the GSI was slighty increased 2.02±0.032 to 2.23±0.015, the preparatory phase follows the pre-spawning phase or maturity stage of reproduction which occurs from May to June month when the GSI of fish was increasing from the previous month (3.2±0.01 to 3.93±0.015). The gonadosomatic index was significantly different during the annual reproductive phases of *Ompok bimaculatus* ($F=1175.45$; $p < 0.01$).

In comparison to female, male had lower GSI. In the case of male, it was found that the gonadal weight was gradually increased from February to July month. The gonadal weight was slowly increased stage by stage until it reached its maximum value in July and August. The highest GSI value was found in the month of August and GSI values began to fall gradually from September to December month, while in female *O. bimaculatus*, the gonadal weight was gradually increased from January to August month. The higher value of GSI for both male and female during July to August month indicated that fish breed during this rainy season.

3.4. Correlation between Gonadosomatic index and different physic-chemical characteristics of Gomati River water

The gonadosomatic index of both male and female showed the same pattern of fluctuations in different months of reproduction. There was a highly significant correlation between male and female GSI with different physico-chemical characteristics of Gomati River water viz., water temperature, pH, total hardness, alkalinity, dissolved oxygen and free CO_2 (Table 9) which was analysed by Pearson correlation by IBM SPSS (version 20.0). The findings of this study was resulted that all the physico-chemical characteristics of Gomati River water were significantly correlate with the gonadosomatic index of male and female *Ompok bimaculatus.*

3.5. Annual morphology of Male

Testes of freshwater butter catfish, *Ompok bimaculatus* exhibited five phases in an annual reproductive cycle (Table 10). The testes of *O. bimaculatus* remain attached to the body wall by means of mesenteries. Each testis was surrounded by an outer thin peritoneum beneath which there was an inner tunica albuginea which was consisted of dense collagenous connective tissue with few fine fibres, they extended inside the testis to give connective tissue septa (Figure 20). The tunica albuginea was covered externally by mesothelium. The testes were composed of seminiferous tubules. characterized with germinal epithelium and central lumen. The seminiferous tubules were filled with

germinal cysts. The germinal cysts contained germinal cells at different stages of development. The testicular lobules were larger and were distended with different spermatogenic stages with the appearance of huge number of spermatozoa in their lumen. The interstitium filled the intertubular spaces and was composed mainly of fine collagenous connective tissue which contained abundant interstitial leydig cells and blood capillaries. The interstitial leydig cells were polygonal in shape, with ill definite cell boundaries. They had centrally located spherical nuclei. The testicular tubules contained two types of cells; one of them was spermatogonia which give rise to different generation of spermatogenic cells in germinal cyst, while the other was Sertoli cells. These cells had flattened and elongated dense nuclei. They surrounded the germinal cysts as their cytoplasmic processes shared in formation of the wall of germinal cysts.

3.5.1. Histological study of freshwater butter catfish, Ompok bimaculatus testes

Five types of germ cells or spermatogenic cells viz, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa were identified in the seminiferous tubules during different reproductive phases (Figure 20, 22).

3.5.1.1. Spermatogonia

These were the largest of all the spermatogenic cells, forming nest and attached to the inner margin of the lobule boundary wall. Each spermatogonium contained prominent cytoplasm and centrally placed nucleus.

3.5.1.2. Primary spermatocyte

The primary spermatocytes contained relatively lesser amount of cytoplasm and the nucleus was deeply stained with haematoxyline. They were found in germinal cysts. They were smaller than spermatogonia. These cells were almost oval or rounded and diameter varies.

3.5.1.3. Secondary spermatocyte

The secondary spermatocytes were arised from the division of the primary spermatocytes. They were smaller than the primary spermatocytes and nearly spherical in shape. They characterized by their large nuclei occupying most of the cells that were surrounded with thin rim of cytoplasm. Their nuclei had condensed chromatin. Secondary spermatocytes were difficult to be observed.

3.5.1.4. Spermatids

The spermatids were smaller than the previous germ cells. They were observed in the cysts. These cells were deeply stained with haematoxyline. They were characterized as appeared as small cells of indistinct outline with scanty cytoplasm and dense spherical basophilic nuclei. Some cysts of spermatids get ruptured and the spermatids released into the tubular lumen.

3.5.1.5. Spermatozoa

These were the eventual consequence of spermatogenesis and smallest one of all spermatogenic lineages. It had strong affinity to haematoxyline. They were present in the lumen of the seminiferous tubules without any arrangement, after their releasing from the germinal cysts of the spermatids.

3.5.1.6. Interstitial cells

The interstitial cells were round or oval in shape and reside singly or in small groups between the lobular spaces and associated with the blood vessels. These cells undergo changes in their morphology in different seasons.

3.5.2. Cyclical changes during spermatogenesis

On the basis of gonadosomatic index (GSI) and the occurrence of various spermatogenic cells, the reproductive phases of *O. bimaculatus* can be dissevered in four

phases: immature or resting phase (November to February month), early development or preparatory phase (March to April month), maturing or pre-spawning phase (May to June month), ripe or spawning phase (July to August month) and spent or post spawning (September to October month) (Figure 20, 22).

3.5.2.1. Immature or resting phase

During immature phase, the predominant spermatogonia were arranged in a definite pattern and few spermatocytes were also present in between them. These stages of cells were present during November to February month.

In November, the mean GSI value was increased insignificantly and maintained as such up to January (Figure 19A). During this phase testis was pale colored and in regressed stage (Figure 20). During these months, histologically, testis was rich in interstitial cells, spermatogonia and spermatocytes. Texture wise arrangement was loose (Figure 22). It represented the resting phase of annual cycle.

3.5.2.2. Early development or preparatory phase

This phase was observed during March to April month. In the early development phase, the testis was characterized by the presence of all stages of the spermatogenic cells and few spermatogonia. The primary and secondary spermatocytes were gradually increased in number.

During February-March, GSI value was recorded significantly increased and become high in April month (Figure 19A).The same reflected by morphological assessment also. During these months testis registered an increase in their size with bright color (Figure 20, 22). Histological sections showed that during this period testis registered a significant increase in spermatocyte. The seminiferous tubules were filled with spermatogonia and spermatocytes (Figure 22). The interstitium was filled the inter-tubular spaces and was composed mainly of fine collagenous connective tissue which

contained abundant interstitial leydig cells and blood capillaries. It represented the preparatory phase of annual cycle.

3.5.2.3. Maturing or Pre-spawning phase

This phase was studied during May to June month. The lobule boundary wall of the testis had become considerably thin and spermatogonia cells were reduced in number or absent. The spermatocytes were reduced considerably and gradually transformed into the spermatids and spermatozoa. The active interstitial cells were noticed in between the lobules.

From April month onwards GSI was gradually increased up to July month. This GSI was increase from April to July was non-significant (Figure 19A). Morphologically during this phase testis was prepared as more flabby and bright colored. It showed clear lobular structure of testis. Histologically testes were rich in spermatocyte, spermatogonia and spermatid.

3.5.2.4. Ripe or spawning phase

The lobule boundary wall was extremely thin and the spermatogenic activity within the lobules was at their peak. The spermatogonia were reduced in number. The testicular lobules were full of spermatozoa and the maximum activity of interstitial cells can be seen in this stage adjacent to blood vessels as they increase in size. This phase was occurred in July to August month.

In July month, testes along with other spermatozoa stage were also seen. The interstitium filled the inter-tubular spaces and was composed mainly of fine collagenous connective tissue which contained abundant interstitial leydig cells and blood capillaries (Figure 22). It represented the pre-spawning phase of annual cycle. GSI value was raised sharply and attained its peak value in August month (Figure 19A). Morphologically also this was very dominating phase. Testis was full grown, lobular, thick and bright colored organ (Figure 20). Histologically this phase testis is full of spermatozoa. With mild

touching it was ready to come out. The testicular tubules were larger and were distended with different spermatogenic stages with the appearance of huge number of spermatozoa in their lumen (Figure 22). It represented the spawning phase of annual reproductive cycle.

3.5.2.5. Spent or post spawning phase

The diameter of lobules was decreased due to release of sperms and boundary wall gradually become thicker. The spermatogonial cells were present in clusters. The testicular lobules contained residual spermatozoa and few cysts of spermatids. The interstitial cells were considerably smaller in size. This phase was occurred in September to October month.

The sharp decrease in GSI value was noticed at the end of spawning phase in September that maintained up to October (Figure 19A). Morphologically also testis appeared shrunken form, that reflected the discharge of mature gamete, spermatozoa (Figure 20). This spent phase was also supported by histological analysis, where sections showed the presence of remnant of interstitial cells and muscular meshwork only (Figure 22).

3.6. Annual morphology of female reproductive organ of freshwater butter catfish, Ompok bimaculatus

3.6.1. Annual macroscopic features of the ovary with oocyte diameter

The ovary was two-lobed and elongated structure. It was attached to abdomen by dorsal mesenteric and extended posterior in to oviduct (Figure 21). The reproductive cycle of *O. bimaculatus* ovary was categorized into five maturation phases on the basis of its macroscopic condition. The relative abundances or monthly changes of percentage frequency of maturity stages of oocytes in the ovary were shown in Figure 25. The division criteria of different maturity stages of freshwater butter catfish, *Ompok bimaculatus* ovary was summarized below in table 11.

3.6.1.1. Immature stage / Resting phase

The ovary was small, thread like in structure, semi-transparent, pinkish in color. The oocytes cannot be seen by naked eyes (Figure 21). Immature ovary was found from the month of November to February. The major event in this phase was proliferation of oogonia and recruitment of oocytes. The average oocytes diameter was less than 0.085±0.002 to 0.112±0.007µm (Figure 24).

3.6.1.2. Early development / Preparatory phase

In early development phase, ovary was elongated and reddish in color. Oocytes were not distinctly visible by naked eye. The thickness of ovary was slightly greater than in immature phase. This phase of ovary was found in March and April month (Figure 21). The oocytes were of primary growth phase, spherical and small of 0.14±0.006 to 0.48±0.032 µm diameter with strongly basophilic cytoplasm (Figure 24). During this phase, ovary occupy one-fourth of the ventral cavity.

3.6.1.3. Maturing stage / Pre-spawning phase

The maturing phase of reproduction of *Ompok bimaculatus* was found during May and June month. In this period, ovary was enlarged in size, reddish in color and had translucent oocytes which were visible by naked eye. The vascularization of ovary was increasingly started. Ovaries occupied less than one-third of ventral cavity (Figure 21). The oocytes were almost round in shape and size of oocytes (0.608±0.019 to 0.672±0.02 µm) was increase due to the yolk vesicles and deposition of yolk droplets (Figure 23, 24).

3.6.1.4. Ripe stage / Spawning phase

Ovaries were highly vascularized and full of mature oocytes. This stage was observed in July to August month. Ovary was in brown in color. The oocyte size was also increased due to hydration and vitellogenesis (Figure 21). The oocyte diameters were varied from 0.795±0.025 to 0.84±0.024 µm (Figure 24).

3.6.1.5. Spent stage / Post-spawning phase

The ovary of the spent stage or post-spawning stage was occurred in September to October month. It were shrunken containing a few atretic oocytes or fully empty. Ovary weight was decreased (Figure 21). Average oocyte diameter was sharply declined (0.052±0.005 to 0.065±0.006 µm) (Figure 24).

3.6.2. Study of oocyte developmental stages

Several stages of oocyte development were observed from October 2014 to September 2015 although ovaries had oocyte of more than two stage of development. The ovaries were surrounded by germinal cuboidal epithelium. There was a connective tissue called tunica albugina which in turn surrounded by ovarian stoma at which female cell was embedded (Figure 23). The histological studies revealed that freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) had different stages of oocyte development.

3.6.2.1. First Growth Phase (Oogonia)

Oogonia were arranged in a single or clusters. The oogonia were very small round cells (0.09 µm) with a clear cytoplasm and a single nucleolus in a oval nucleus and had chromophobic cells (Figure 23).

3.6.2.2. Second Growth Phase (Chromatin nucleolus stage)

The cell diameter of this oocyte was 0.14 µm. These oocytes were originated from oogonia. They had little cytoplasm large and stained nucleus and central nuclei. This oocyte was basophilic in nature (Figure 23).

3.6.2.3. Third Growth Phase (Early peri-nucleolus oocyte)

These oocytes were larger oocyte (0.32 µm) in relation to earlier oocyte. It has homogeneous and strongly basophilic cytoplasm; numerous rounded, basophilic nucleoli appeared at the periphery of the nucleolus (Figure 23).

3.6.2.4. Forth Growth Phase (Late perinucleolus oocyte)

The oocytes (0.61 µm) showed low affinity to haematoxyline. Numerous rounded nucleoli were found in the periphery of the nucleolus the cytoplasm was dense. A flattened follicular layer surrounding oocytes could be distinguished at the end of this stage (Figure 23).

3.6.2.5. Fifth Growth Phase (Yolk Vesicle Stage)

These oocytes were spherical (0.72 µm) in appearance during this oocyte development stage. Cortical vesicle appeared in the cytoplasm. These vesicles were first situated at the cytoplasm periphery and then subsequently moved to the inner cell zone. The cytoplasm stained lighter and nucleus become increasingly eosinophilic. Zona radiata was formed between the oocyte and its follicle layer. In the late yolk stage the protein yolk granule was filled equal to or greater than two-third of the cytoplasm. The oocyte and nucleus reached at maximum size, Granular cells of follicle layer appeared stretch and become thin. The nucleus was smaller and compared to the earlier stages. Chromatin threads still occurred in the nucleus, and the nuclear membrane began to degenerate. The zona radiate layer was very evident and follicle epithilium was more developed (Figure 23).

3.6.2.6. Sixth Growth Phase (Vitellogenesis)

The oocytes were 0.88 µm in size. It began to undergo vitellogenesis. In this stage, the oocyte appeared to be pinkish red, the yolk vesicle and granules were observed in whole cytoplasmic area. The nuclear membrane was completely degenerated. The follicular layer and zona radiate were now well developed and distinct.

3.6.2.7. Seventh Growth Phase (Vitellogenic)

These oocytes were largest in size (0.96 µm diameter) and completely filled with protein yolk granule. The cytoplasm was more voluminous and had grainy appearance. At this stage the nucleus shifted towards the oocyte periphery. The succeeding nucleus migration was the GVBD (Figure 23).

3.6.2.8. Eight Growth Phase (Post ovulatory follicle)

After maturity, the follicles breakdown to allow oocyte released post-ovulatory follicle (diameter: 0.08 µm) consisted of residual follicle layers, which remained in the ovaries after ovulation and degenerated.

3.6.2.9. Atretic Follicle

After final spawning a number of post-vitellogenic oocyte failed to undergo maturation or ovulation and subsequently degenerated and were reabsorbed that was become atretic. The diameter of atretic follicle was 0.04 µm. At this stage zona radiate become wrinkled and standard to breakdown (α Atretia). The disordered yolk liquefied and cytoplasm and proliferated phagocytic granulosa cells were found, which become degenerated and living behind a lightly stained fibrous mass surrounded by connective tissue element (β atretia) (Figure 23).

3.6.3. Oocyte diameter

The oocyte diameter (OD) showed the same pattern in size difference as like of GSI in different months. The mean OD was ranged from 0.085±0.002 µm (immature stage) to 0.84±0.024 µm (mature stage) (Figure 24). OD was significantly different during the sampled months ($F=432.035$; $p < 0.001$). The OD was minimum during September to January months (0.052±0.005 to 0.089±0.004 µm respectively). Thereafter a slight but non-significant increase was noticed during February when compared with January (0.1125±0.007). A significant increase in OD was seen during March month

(0.48±0.032 µm; $p < 0.01$).That maintained in sharp significant increase from previous months to April onwards ($p < 0.01$) with peak value in August (0.84±0.024 µm).

There was a highly significant correlation between gonadosomatic index (GSI) and oocyte diameter (OD) during different months (Table 12). The coefficient of determination and regression equation between the GSI and OD was r^2=0.771 and y=0.1667X+0 respectively (Figure 26). Both these two parameters GSI and OD supported five phases of annual reproductive cycle of freshwater butter catfish, *Ompok bimaculatus* ovary, like: November, December and January: resting phase; February, March and April: preparatory phase; May, June and July: pre-spawning phase; August: spawning phase; September and October: post spawning phase.

Table 8: Showing pearson correlation of monthly changes in physico-chemical properties of Gomati River water (Alk: Alkalinity, DO: Dissolved oxygen, Free CO_2: Free carbon dioxide, pH, T: Temperature, TH: Total hardness). Asterisks (*) represented the significance level.

Correlations		**Alk.**	**DO**	**Free CO_2**	**pH**	**T**	**TH**
Alk.	Pearson Correlation	1	0.620*	0.725**	0.125	0.624*	0.074
	Sig. (2-tailed)		0.032	0.008	0.699	0.03	0.818
DO	Pearson Correlation	0.620*	1	0.559	0.555	0.755**	0.378
	Sig. (2-tailed)	0.032		0.059	0.061	0.005	0.226
Free CO_2	Pearson Correlation	0.725**	0.559	1	0.606*	0.884**	0.64
	Sig. (2-tailed)	0.008	0.059		0.037	0.00	0.025
pH	Pearson Correlation	0.125	0.555	0.606*	1	0.761**	0.845**
	Sig. (2-tailed)	0.699	0.061	0.037		0.004	0.001
T	Pearson Correlation	0.624*	0.755**	0.884**	0.761**	1	0.755**
	Sig. (2-tailed)	0.03	0.005	0.00	0.004		0.005
TH	Pearson Correlation	0.074	0.378	0.64	0.845**	0.755**	1
	Sig. (2-tailed)	0.818	0.226	0.025	0.001	0.005	

****** Correlation is significant at the level of 0.01 (2-tailed).

***** Correlation is significant at the level of 0.05 (2-tailed).

Table 9: Showing pearson correlations of annual male and female gonadosomatic index of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) with different physico-chemical properties of Gomati River water (T: Temperature, TH: Total hardness, Alkalinity, BOD: Biological oxygen demand, DO: Dissolved oxygen). Asterisks (*) represented the significance level.

Correlations		**Alkalinity**	**DO**	**Free CO2**	**pH**	**T**	**TH**
Female GSI	Pearson Correlation	0.428	0.889**	0.455	0.547	0.595*	0.379
	Sig.(2-tailed)	0.165	0.000	0.137	0.066	0.041	0.224
Male GSI	Pearson Correlation	0.305	0.809**	0.558	0.718*	0.732*	0.677*
	Sig.(2-tailed)	0.335	0.001	0.06	0.008	0.007	0.016

****** Correlation is significant at the level of 0.01 (2-tailed).

***** Correlation is significant at the level of 0.05 (2-tailed).

Table 10: Macroscopic features and description of maturity stages of testes of freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794) sampled from Gomati River.

Months	**Maturity Stages / Reproductive phase**	**Macroscopic description**	**Developmental description**
November December January February	Immature / Resting phase	Thin, whitish thread-like structures, very small and translucent	Predominant spermatogonia were arranged in a definite pattern and few spermatocytes present
March April	Early development / Preparatory phase	Aspect of ribbon, slight pinkish color, turgid and voluminous	Primary and secondary spermatocytes were gradually increased in number
May June	Maturing / Pre-spawning phase	Ribbon shaped, slightly red in color and with flaccid part.	Spermatogonia cells were reduced and gradually transformed into the spermatids and spermatozoa.
July August	Ripe / Spawning phase	Ribbon shaped, increase in size from earlier stage, slightly red in color and flaccid	Spermatogenic activity was at their peak. testicular lobules were full of spermatozoa
September October	Spent / Post-spawning phase	Thread shaped, thin and slight pink in color	Spermatogonial cells were present in clusters. Lobules contained residual spermatozoa and few cysts of spermatids.

Table 11: Macroscopic features and oocyte developmental descriptions of maturity stages of ovary of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from Gomati river, Lucknow, U.P.

Months	**Maturity Stages / Reproductive phase**	**Macroscopic description**	**Oocyte developmental description**
November December January February	Immature / Resting phase	Small in size, semi transparent, pinkish in color	Containing both oogonia and chromatin nucleolus and early peri-nucleolus stage
March April	Early development / Preparatory phase	Ovaries occupied one-fourth of the ventral cavity, reddish in colour	Oognia still present, late peri-nucleolus and yolk vesicle or corticle alveoli oocytes present
May June	Maturing / Prespawning phase	Ovaries occupied less than one-third of the ventral cavity and opaque and translucent oocyte visible, increasingly vascularization	Yolk granule oocytes predominate, late peri-nucleolus and vitellogenic oocyte also be observed
July August	Ripe / Spawning phase	Ovaries highly vascularized and occupied most of the ventral cavity, distinctly and lobular in appearance, brown in colour, large oocyte, yolky	Vitellogenic oocyte present, predominance of germinal vesicle migratory oocyte

Contd…

		and eggs excruted with slight abdominal pressure	
September October	Spent / Post-spawning phase	Ovaries flaccid and fully empty, slightly brown in colour, hyaline oocyte visible, granular small ovaries with scattered residual vitellogenic and atretic oocytes	Abundant post-ovulatory follicle, peri-nucleolar oocytes and atretic oocyte present

Table 12: Showing pearson correlations between monthly changes in Gonadosomatic index and oocyte diameter (µm) of freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794). Asterisks (*) represented the significance level.

Correlations		Oocyte Diameter
Gonadosomatic index	Pearson Correlation	.884***
	Sig. (2-tailed)	.000
***. Correlation is highly significant at the 0.01 level (2-tailed).		

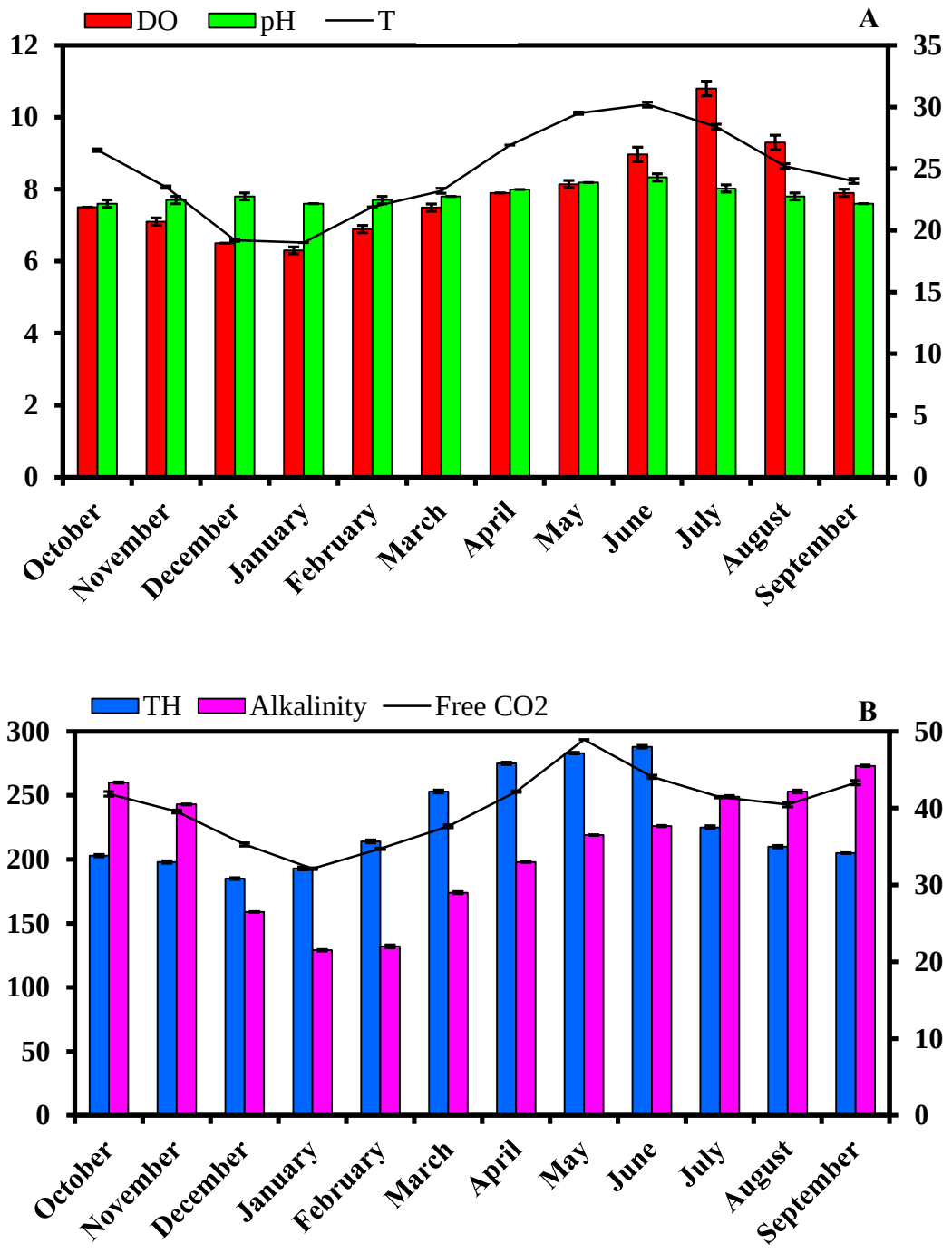

Figure 18: Represent annual variations in physico-chemical characteristics of Gomati River water during observational period from October 2014 to September 2015. Graph showed DO (Dissolved oxygen: mg/l), pH, T (Temperature: ^{o}C), TH (Total hardness: mg/l), Alkalinity (mg/l) and Free CO_2 (Free carbon dioxide: mg/l).

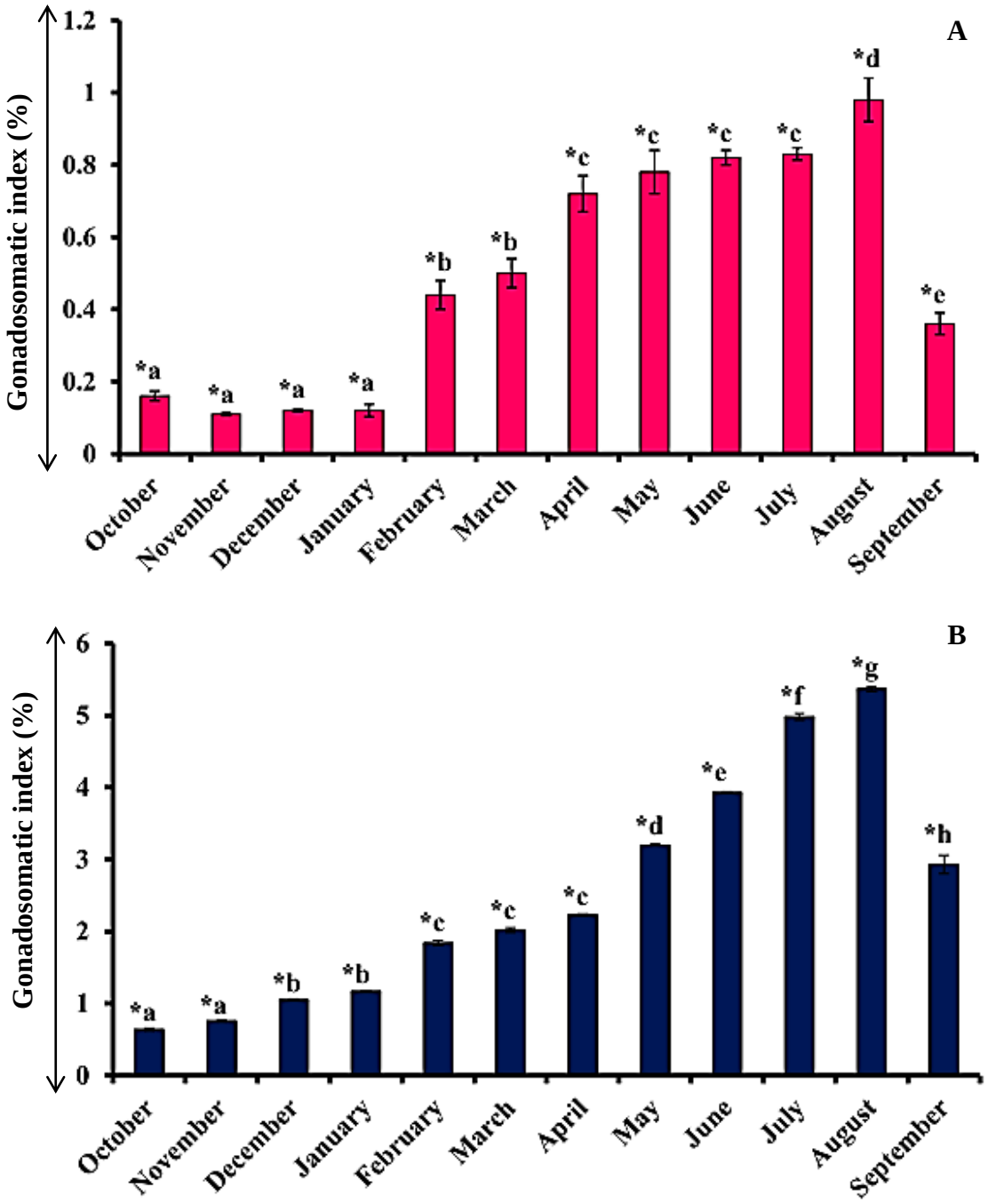

Figure 19: Showing the annual changes in gonadosomatic index (GSI) (%) of male (A) and female (B) freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794). Values were expressed as mean±SEM. Data were analyzed by one way ANOVA ($p < 0.001$) and intergroup difference were analyzed by Neuman-Keuls test ($p < 0.05$). Same alphabets represented non-significant difference but different alphabets represented significant difference respectively.

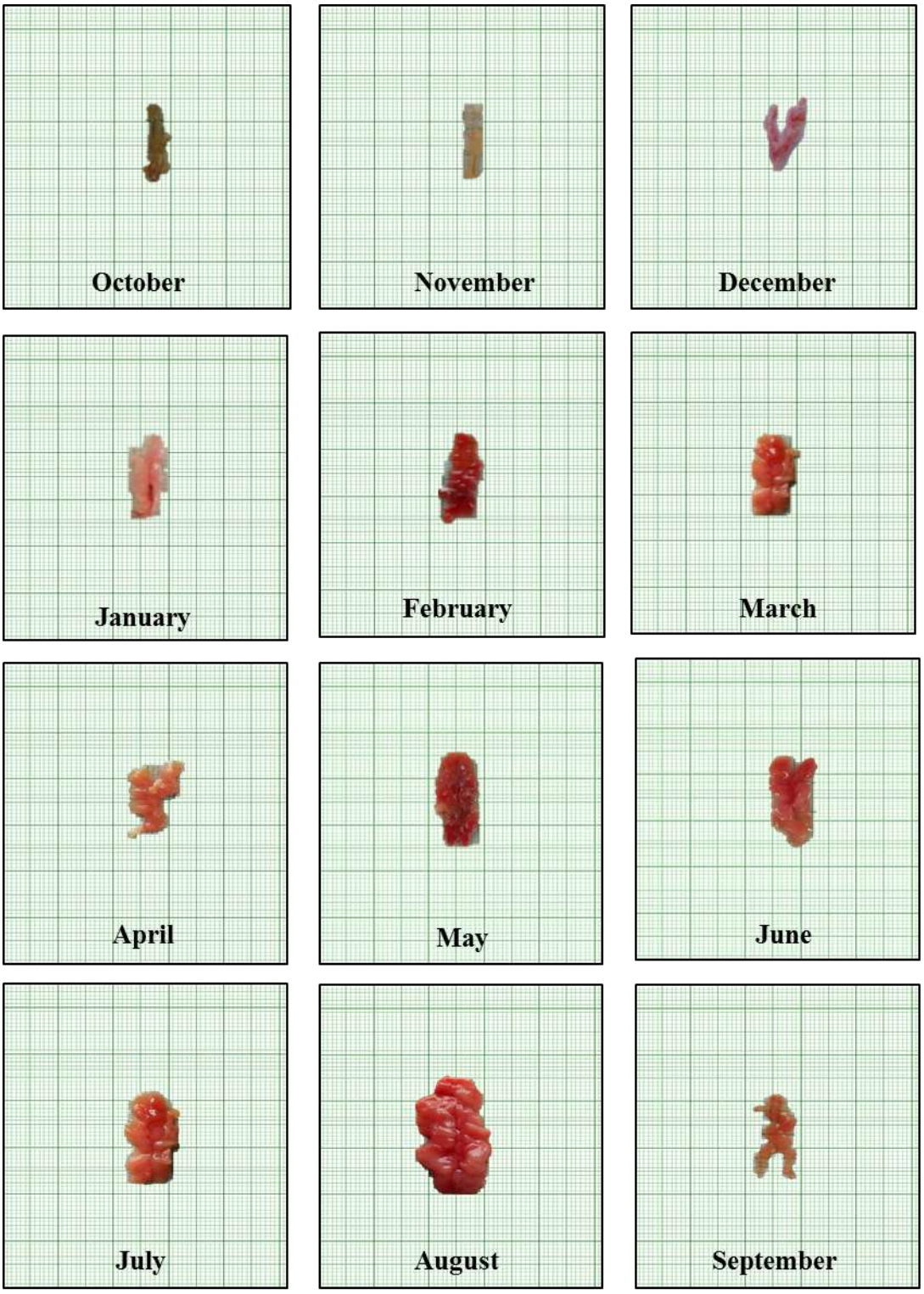

Figure 20: Annual morphological appearance of testes of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from Gomati River, Lucknow, during October 2014 to September 2015 period.

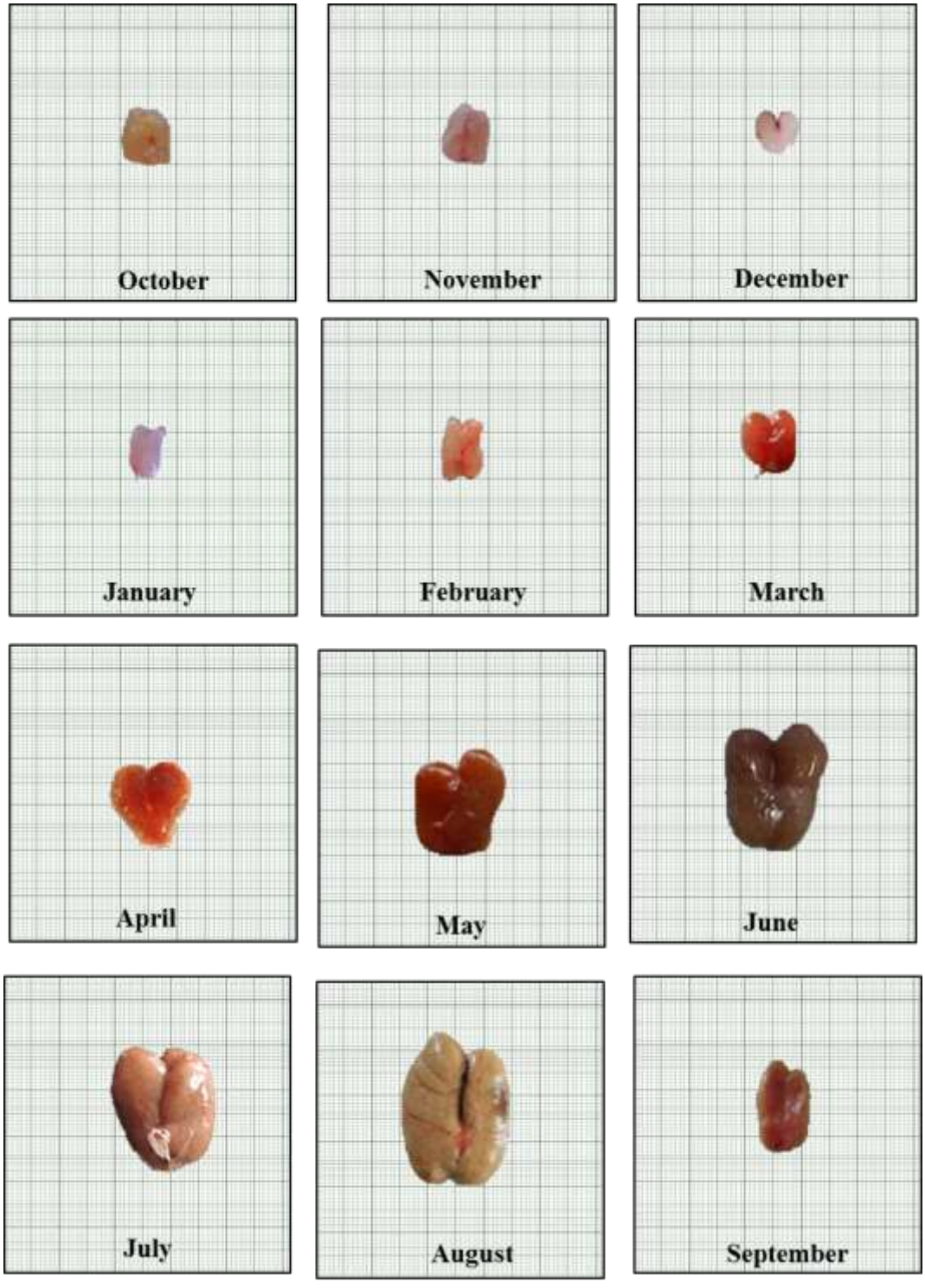

Figure 21: Annual morphological appearance of ovary of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from Gomati River, Lucknow, during the period of October 2014 to September 2015.

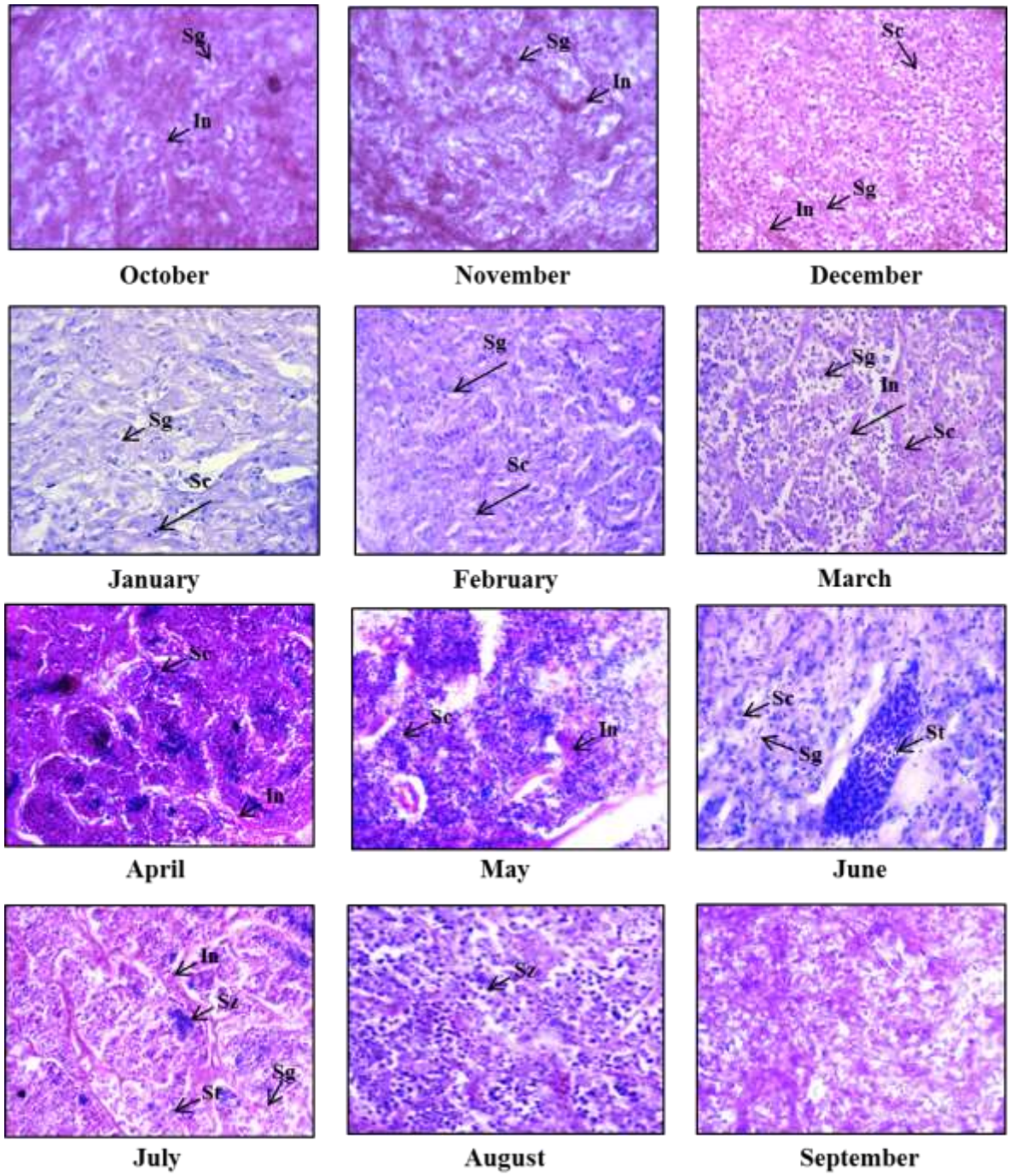

Figure 22: The histological sections of testes of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled during different months from October 2014 to September 2015. Images were captured at 20X magnification. Note: In: Interstitial cells, Sg: Spermatogonia, Sc: Spermatocytes, St: Spermatids, Sz: Spermatozoa.

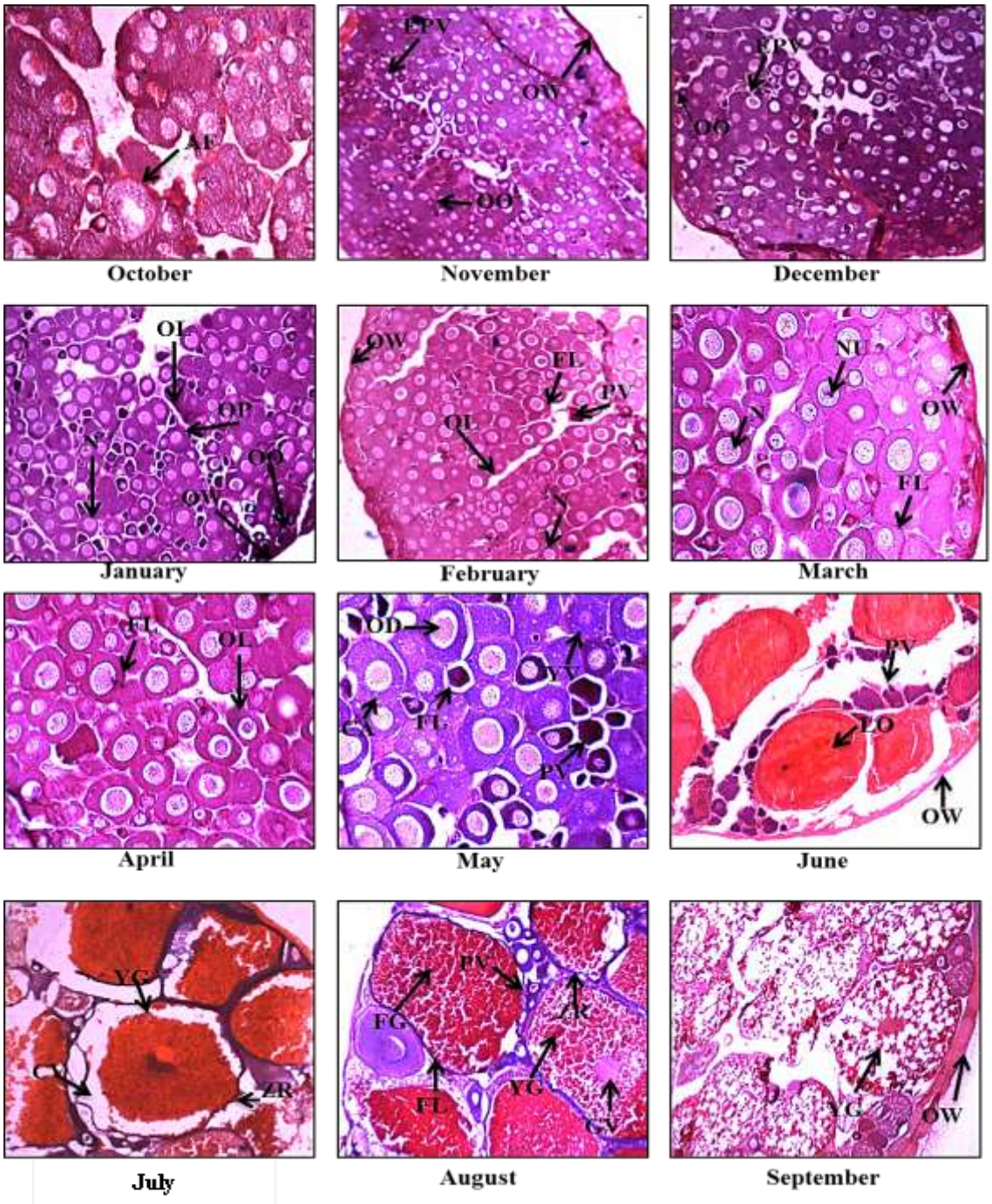

Figure 23: Histological appearance of ovary of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) during different months from October 2014 to September 2015. Images were captured with 20X magnification. Note: OL: Ovarian lumen, OO: Oogonia, OW: Ovarian wall, N: Nucleus, OP: Ovarian peritoneum, FL: Follicle layer, PV: Pre-vitellogenic oocyte, GF: Graffian follicle, AF: Atretic follicle, NU: Nucleolus, YV: Yolk vesicle, LP: Late peri-nucleolus, CA: Corticle alveoli, EPV: Early peri-nucleolus vesicle, YG: Yolk globule, ZR: Zona radiata, GV: Germinal vesicle.

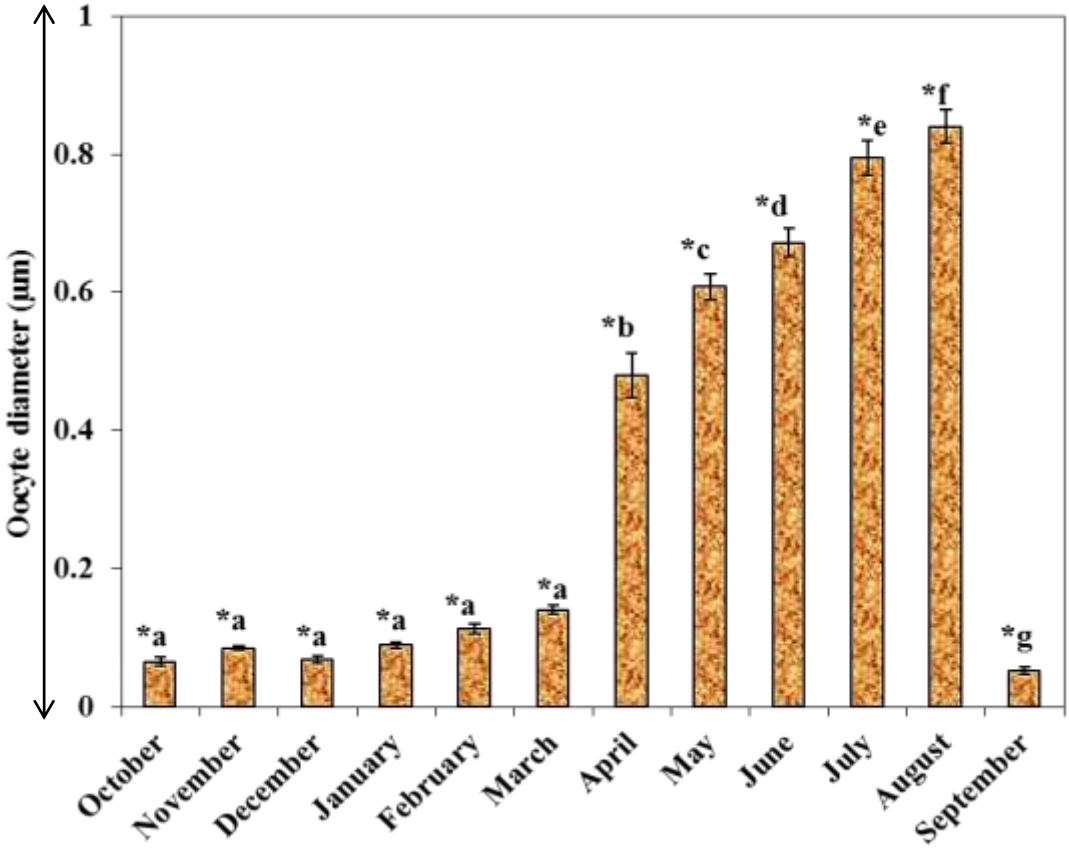

Figure 24: Showing the monthly changes in oocyte diameter (µm) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) ovary. Values were expressed as mean±SEM. Data were analyzed by one way ANOVA ($p < 0.001$) and intergroup difference were analyzed by Newman-Keuls test ($p < 0.05$). Same alphabets represented non-significant difference but different alphabets represented significant difference among groups in oocyte diameter respectively.

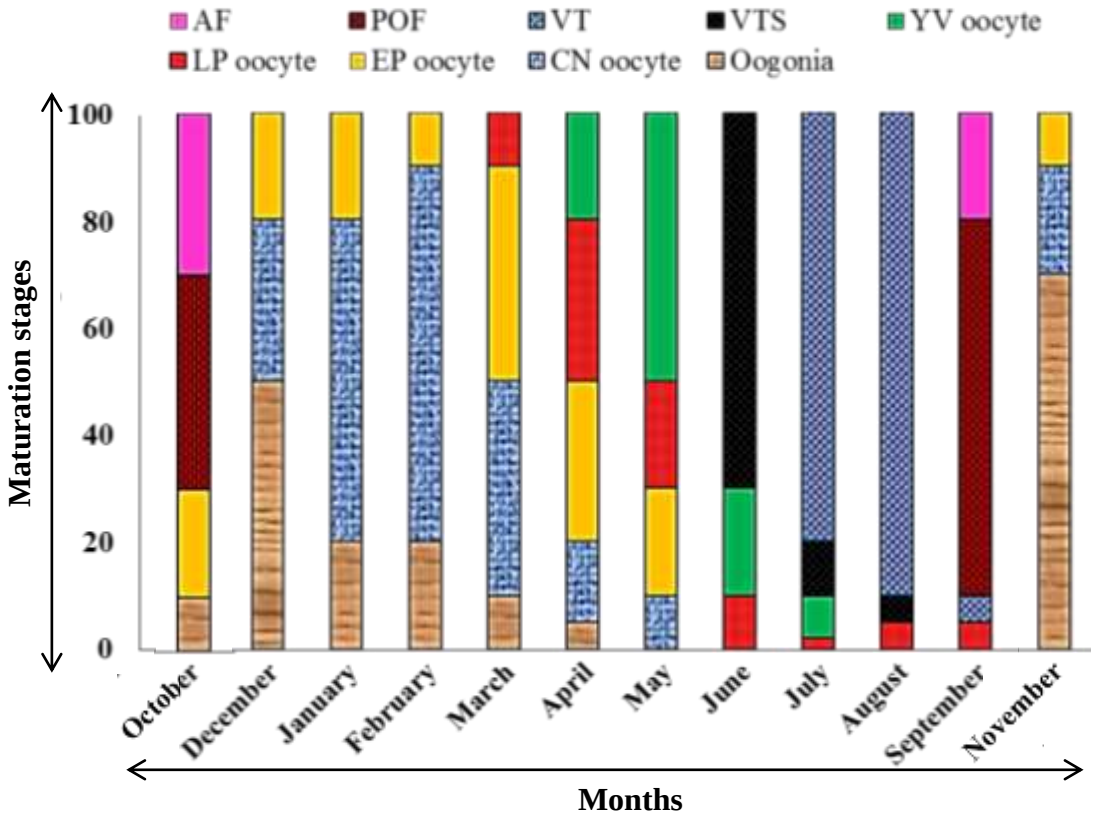

Figure 25: Monthly changes in frequency (%) of oocyte maturation stages of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in histological sections. AF: Atretic follicles; POF: Post ovulatory follicles; VT: Vitellogenic oocyte; VTS: Vitellogenesis; YV: Yolk vesicle oocyte: LP: Late peri-nucleolus oocyte; EP: Early peri-nucleolus oocyte; CN: Chromatin nucleolus oocyte; Oogonia.

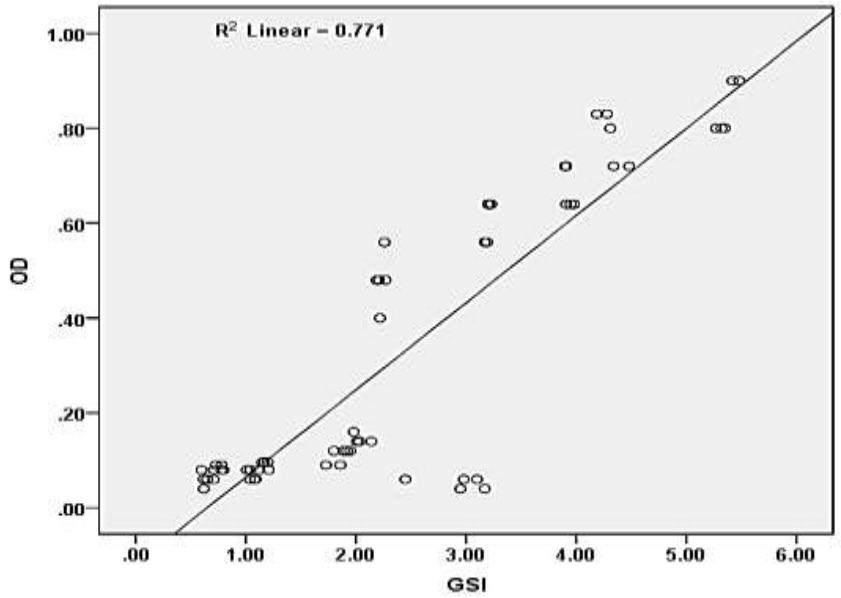

Figure 26: Showing correlation between gonadosomatic index (GSI) and oocyte diameter (OD) of different maturity stages of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

4. Discussion

The present study was described about the annual reproductive cycle of male and female freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) collected from Gomati River, Lucknow. This freshwater catfish of India exhibit the annual periodicity of gonadal maturation and spawning which occurred in a particular period of the year. The process of gonadal recrudescence is positively correlated with the atmospheric seasons (Gonzalez, 1980; Gentile et al., 1986; Guerrero et al., 1990). The onset of the rainy season provides favorable environmental conditions to promote spawning and spermiation. Spawning, which is completed in a very short time, is followed by a period of sexual quiescence during the rainy season.

The meteorological observation resulted that monthly rainfall was fluctuated from 0 to near 100 mm. Air temperature was varied over a range of about twenty degrees (from 13.98 to 33.36 ^{o}C). The highest temperature were observed from April to June (dry season), decreasing by July (rainy season). Studies on various teleost species indicated that temperature was a primary environmental cue that regulates gonadal recrudescence and spawning (Lam, 1983; MacKenzie et al., 1989; Peter and Yu, 1997). The observation of Acharia et al. (2000) supported the phenomenon of inducing ovarian recrudescence during the late post-spawning phase under high temperature in the case of female *Clarias batrachus*. Increase in photoperiod and temperature promote gonadal maturation and so the breeding response (Sathyanesan, 1962; Sundararaj and Vasal, 1976; Garg and Jain, 1984; Miranda et al., 2009). Spawning was occurred during the period of maximum rainfall or in rainy season and low air temperature (Gentile et al., 1986; Ntiba and Jaccarini, 1990; Villacorta-Correa and Saint-Paul, 1999; Mazzoni et al., 2002; Agostinho, 2003; Marcano et al., 2007). Most likely, spawning could be triggered not by one external condition, but by a combination of several. The spawning season was inferred from the relative frequencies of gonad maturity stages throughout the study period.

Gonadosomatic index (GSI) is a most reliable reproductive measure when associated with other indicators as gonadal macroscopic and microscopic observations

and oocyte diameter to study reproductive cycle (Lowe-McConnell, 1982; Rimmer and Merrick, 1983; DeMartini and Lau, 1999). The result showed that GSI had a positive relationship with the gonadal developmental stages of ovary and spermatogenic activity in the testis. Similar findings were reported by different workers time to time (Mukhopadhyay and Sinha, 1986; Joshi and Joshi, 1989; Stoumbondi et al., 1993; Chakrabarti and Gupta, 1994; Rheman et al., 2002; Manna et al., 2010; Chakrabarti and Bose, 2014). GSI was varied accordingly during the different months of the year. The values of gonadosomatic index (GSI) were low from December to April month suggesting the ovaries to be in resting and maturing stages. The values increased in June (male: 0.82 and female: 3.93) and reached in peak in August (male: 0.98 and female: 5.37) indicating the gonads to be in mature and ripe stages. The low values from September to October suggested the spent condition or post-spawning phase (Male: 0.36, Female: 2.93) when the spermatogenic and oogenic activities were almost ceased. Fish attain maturity in one year in Gomati River. On the basis of GSI value it has been observed that peak level of GSI in the month of July and August, were closely related with the maturity and spermiation (Banik et al., 2012; Mishra et al., 2013; Malla and Banik, 2015). The GSI and oocyte diameter increased with that of maturation stage of fish. It reached its maximum at the peak of maturity and at minimum during resting phase of ovary (Shengde and Mane, 2006; Rao and Krishnan, 2009; Ghanbahadur and Ghanbahadur, 2012; Mishra and Saksena, 2012).

The present morphological and histological studies of gonads (Ovary and testis) had revealed that the gonads of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) exhibited remarkable variations in their size. Prior to spawn the testes and ovaries undergo preparatory and pre-spawning stages which included various degree of developmental changes in gamete related to spermatogenic and oogenic activity. This would further change the gonadal texture, shape and size. On the basis of spermatogenic and oogenic activity and variations in GSI values obtained in the present study the entire reproductive cycle is divided into five distinct phases' viz., resting (November-January), preparatory (February-April), pre-spawning (May-July), spawning (August) and post-spawning (September-October). All parameters of current study viz. GSI, oocyte

diameter, different oocyte and testicular stages and macro-, micro-scopic gonadal details strengthen this statement of reproductive phases of freshwater butter catfish *Ompok bimaculatus* (Bloch, 1794).

The phenomenal variation in the testis was due to active proliferation of the later stages of spermatogenetic cells. The spermatozoa were formed from spermatogonia through a series of cytological changes known as spermatogenesis. In the present investigation the dormant nests of spermatogonia occurred in the post-spawning and resting period (Malla and Banik, 2015). During the process of differentiation of spermatogonia to spermatozoa, the cytoplasm and nuclei of spermatogonia progressively decreased in size and volume, and finally the spermatids were metamorphosed into spermatozoa (Chaves-Pozo et al., 2005; Suwanjarat et al., 2005; Van Dyk and Pieterse, 2008). In the present study preparatory phase was characterized by maximum number of spermatogonial cells. However, gradual cellular activity was found to be associated with the testicular lobules during the early growth phase which in turn was characterized by an increased activity in the conversion of spermatogonia to primary and secondary spermatocytes, few spermatids and spermatozoa. The number of spermatocytes increased gradually reaching the maximum number in the pre-spawning phase. However, during the end of this phase the secondary spermatocytes were rarely seen and enormous number of cysts of spermatids and spermatozoa were almost completely filled up the entire lumen of testicular lobules (Dziewulska and Domagala, 2003). The spawning phase was characterized by the presence of maximum number of spermatozoa and spermatids in the testicular lobules. This was due to the rapid spermiogenesis, during this phase (Dziewulska and Domagala, 2003). The spermatogenic activity was decreased sharply following the regressive period and the testes finally entered into the post-spawning phase. This phase was characterized by almost empty containing residual spermatozoa and few cysts of spermatids along with few dormant spermatogonial cells (Suwanjarat et al., 2005; Cek and Yilmaz, 2007; Lawson, 2011; Ahmed et al., 2013).

In teleosts, the annual ovarian development process may be divided into four to eight maturity stages or phases (Sathyanesan, 1962; Guraya et al., 1975; Nagahama,

1983; Mayer et al., 1988; Treasurer, 1990; West, 1990; Fishelson et al., 1996; Unal et al., 1999; Verma, 2013). In female freshwater butter catfish, *Ompok Bimaculatus* (Bloch, 1794), During resting phase (November to January month), ovary had majority of oogonia stage (Htun-Han, 1978a, b). Ovary also represented increasing chromatin nucleolus developmental stage. Chromatin nucleolus oocytes developed to early peri-nucleolus oocyte stage in preparatory phase where this present in majority (Ebisawa, 1990). Results showed that February to April month represented preparatory phase in *O. bimaculatus* as in other catfish of Northern region (Raghuveer and Senthilkumaran, 2010). The period May to July month represented as the rapid growth phase of oocytes so as the ovary. This period showed pre-spawning phase of female *O. bimaculatus* annual reproduction cycle. Vitellogenesis completed in this phase and follicles accumulated yolk to form vitellogenic follicles. Primary oocytes grew rapidly with the incorporation of yolk. In preparatory phase, ovaries were dominated by peri-nucleolar oocyte with large nuclei and many various sized nucleoli. The nucleoli played an important role in vitellogenesis (Malhotra, 1963). The size of nuclei decreased with developing stages of the oocytes. The peri-nucleolar stage of oocyte was further transferred in to yolk vesicle stage. The growth during pre-spawning phase was mainly due to formation of yolk vesicles and deposition of yolk. Yolk vesicles or cortical alveoli were the characteristics feature of vitellogenic oocytes (Guraya, 1993). Same changes have been reported in the ovaries of several teleostean species (Jadhav and Bapat, 1983; Burton and Idler, 1984). Yolk vesicles contained endogenously synthesized lipids and glycoprotein and increased the space for incorporation of exogenously synthesized yolk protein (Guraya, 1986; Selman et al., 1986).The vitelline membrane appeared commonly at the yolk vesicle stage and sometimes at the end of primary oocyte (West, 1990; Unal et al., 1999). The spawning period was the month of August for this fish species. During this period, fish attained maximum ovary weight, and ovary was full of mature vitellogenic follicles. The GSI exhibited increasing trend from February onwards (preparatory phase) and highest in August month (spawning phase) which was the indication of large quantity of yolk accumulation in mature ova (Hoque and Hossain, 1993; Encina and Lorencio, 1997; Roy and Hossain, 2006; Alam and Pathak, 2010). The oocytes diameter was also significantly

increased during this phase. The germinal vesicle migration and breakdown was the important event of oocyte maturation. Yolk globules were coalesced to formed a translucent yolk mass. Furthermore, the cytoplasmic content was diluted due to hydration thus appeared as translucent and maximum size of oocytes (Foucher and Beamish, 1980). This fish showed a small spawning phase different to sister fish *O. pabda* (Siddiqua et al., 2000).

At the end of this phase, the ovary decreased in weight not only due to ovulation or discharge of the eggs, but also due to degeneration of oocytes which was referred to as atresia during September month. This post-spawning phase was supported by low GSI and OD. Similar observations were reported in different fish species (Lehri, 1968; Sivakumaran et al., 2003; Chakraborty et al., 2007). Further GSI was drastically decreased in post-spawning and resting phase during month of September onwards. The oocyte diameter also registered a sharp fall in September to October month due to atretic follicles followed by generation of new oogonia. The lowest value of GSI was a result of intense spawning activity (Adamassu, 1996).

Most of the fish spawn during July month in wild conditions but the present investigation found that the best spawning period for freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) was August. In August month, male and female fish spp. had highest GSI. This gave us a clue regarding nature adjustment for some fish species to be lagging behind during breeding season to reduce interspecies competition for breeding ground and later on for survival of young ones (Malla and Banik, 2015). Monthly study regarding GSI, morphological and anatomical changes of testis and ovary of *O. bimaculatus* from the Gomati River represents a clear support to annual reproductive cycle. This reflected that annual reproductive cycle of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) is a cyclic dynamic process and can be manipulated by internal and external parameters.

Chapter III

Induced breeding performance of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) with different hormones

ABSTRACT

The present study was aimed to provide information about induced breeding and embryonic development of near threatened freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794). Induced breeding of *O. bimaculatus* was performed by three different commercially available hormones viz., Ovidac, Gonopro-FH and Ovaprim. During the experiment, dosage of selected hormones was scanned for successful breeding to get most effective concentrations. Both male and female brooders were injected with a range of hormones in a single dose. The result showed that 15:5 IU/g body weight dose of Ovidac, 0.9:0.2 ml/kg body weight dose of Gonopro-FH and 1.2:0.4 ml/kg body weight dose of Ovaprim in female/male were significantly effective dosage which has given best response regarding number of eggs ovulated (Ovidac: F=255.5, Gonopro-FH: F=370.29, Ovaprim: F=112.54; $p < 0.05$), fertilization rate (Ovidac: F=47.45, Gonopro-FH: F=62.74, Ovaprim: F=64.59; $p < 0.05$) and hatching rate (Ovidac: F=15.3, Gonopro-FH: F=31.21, Ovaprim: F=39.07; $p < 0.05$). In Ovaprim induced group of fish, the Latency period was less (11 hr) as compared to other exogenous hormones viz., Gonopro-FH (12 hr) and Ovidac (14 hr). The highest significant fertilization rate (92.73±0.64%) and hatching rate (89±0.29%) was noticed in effective dose of Ovaprim injected group of fish as compared to Gonopro-FH (76.41±0.87% fertilization rate; 70.76±0.35% hatching rate)

and Ovidac (68.38±0.51% fertilization rate; 62.24±0.41% hatching rate) (F=269.79, F=99.15; $p < 0.05$). The embryonic development was same in all tested induced hormones. By research output It may stated that near threatened freshwater butter catfish, *Ompok bimaculatus* might be breed in captivity like other catfish with Ovaprim which act as a most potent exogenous hormone.

Keywords: Ompok bimaculatus, Ovaprim, Gonopro-FH, Ovidac

1. Introduction

The freshwater butter catfish, *Ompok bimaculatus* is commonly known as pabda. This spp. has great consumer preference due to its high nutritive value and good taste. In general, catfish have an annual reproductive cycle with five to six different reproductive phases viz., resting, preparatory, pre-spawning, spawning, post-spawning and spent phase (Sundararaj and Vasal, 1976; Lamba et al., 1983). *O. bimaculatus* was also considered as an annual breeder (Chakraborty et al., 2010; Malla and Banik, 2015). In natural conditions, they breed during their spawning phase only. Adequate availablilty of fish seed is the basic requirement for aquaculture culturist. But to get a regular supply of seed, fish culturist may induce them before their breeding period for spawning (Surnar et al., 2015). Procurement of pure fish seed from a dependable source posed a problem. Seed availability from wild condition is usually seasonal and depend on several environmental cues, such as fluctuations in food availability, photoperiod, temperature (Bye, 1990) and gonadal recrudescence failure, may occur in cultured fish, thus resulted in decreasing fecundity and gamete quality of brood stock (Crim, 1991). Unlike major carp fish, catfish cannot breed in captivity even after steroid hormone injection (Kiran et al., 2013).

The captive breeding is an essential requirement for mass seed production, culture and management to conserve fish population (Khan and Mollah, 1998; Sarkar et al., 2006; Vijayakumar, 2010; Banik and Malla, 2014). Induced spawning enables brooders to ovulate under intensive culture conditions and allows the eggs. The techniques of induced spawning would help culturists to increase hatchery production. The spawning

knowledge of this species is inadequate in India, especially in the North area. The first attempt in this direction was made by Khan (1938), who tried to induce spawning of *Cirrhina mrigala* by injecting hormones of anterior lobe of mammalian pituitary gland (hypophysation). The earlier pituitary extract was utilized to induced fish for breeding in captivity (Zonneveld et al., 1988, Surnar et al., 2015). With the year, several steroids or hormones and their combinations were used to induce fish for breeding in captivity (Goos et al., 1987; Manickam and Joy, 1989; Surnar et al., 2015). The ever increasing cost of donor pituitary and the cumbersome process obliged researchers to use other alternative synthetic hormones.

Now a day's synthetic hormones and their combinations are available in the market to make fish breed in captivity. Among synthetic hormones, human chorionic gonadotropin (hCG) **(**Mollah and Tan, 1983; Zairin et al., 1992; Inyang and Hettiarachchi, 1994), luteinizing hormone-releasing hormone (Billard et al., 1984a, b; De Leeuw et al., 1985; Fermin, 1992) and ovaprim (Alok et al., 1993; Haniffa et al., 1996) were used for induced breeding and have given good results in breeding performance of fish (Kahkesh et al., 2010; Surnar et al., 2015). To select best one, fish culturist have to pay attention to the latency period, spawning efficiency, fertilization rate and hatchability along with survival rate among offspring. Embryonic development is a complicated process provides exceptional knowledge about the cellular differentiation and proliferation (Marimuthu and Haniffa, 2007). The reproductive biology and developmental study give the baseline information for researchers and fish culturist for its seed production, management and conservation (Chakrabarty et al., 2008). Modern aquaculture aims to provide constant production system with a steady supply of eggs and larvae. In aquaculture, this species did not receive much attention due to the insufficiency of stock and the shortage of information regarding its breeding knowledge (Parameswaran et al., 1970; Banik et al., 2002; Banik and Malla, 2009).

Several studies are available about reproductive cycle and breeding methods of catfish (Sundararaj and Vasal, 1976; Alok et al., 1998; Kiran et al., 2013). But little is available for freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794), and that is also

for restricted regions (Mishra et al., 2013; Malla and Banik, 2015). There are some other reports of the successfully induced breeding of catfish in captivity (Zairin et al., 1992; Alok et al., 1998; Bhowmik et al., 2000; Sarkar et al., 2005; Chakrabarty et al., 2006). Little attempts were made for induced breeding of freshwater butter catfish *Ompok bimaculatus* (Chaudhuri, 1962; Raizada et al., 2013). The objective of the present study was to compare and determine the most appropriate commonly used inducing steroid hormones for spawning of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794). I tried to get the effective dose of inducing steroid hormone for successful breeding for aquaculture management and conservation. Also, the study was planned to provide detailed embryonic development information of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

2. Materials and Methods

2.1. Chemicals

Different inducing steroid hormones in the trade name of Ovidac (human Chorionic Gonadotrophin; Zydus Pharma Pvt. Ltd.), Gonopro-FH (synthetic gonadotropin releasing hormone analogue; APC nutrient Pvt. Ltd.) and Ovaprim (synthetic gonadotropin-releasing hormone analogue and domperidone, Western Chemical Inc.) were purchased locally from Lucknow, Utter Pradesh, India. All other chemicals used in the present experiment were of analytical grade and purchased locally from scientific suppliers.

2.2. Animal collection

The live healthy brooders of freshwater butter catfish, *Ompok bimaculatus* (adult male and female) collected from different sites of the Gomati River, a major tributary of Ganga River Basin, Lucknow, with the help of local fisherman during the month of July-August (early spawning or late pre-spawning phase of the annual reproductive cycle) (Figure 27). After collection, fish were brought to the laboratory in wide mouth plastic

container with proper care and aeration. They were acclimatized in 200 lt glass aquaria containing fresh water having ≈7.5 pH, 5-6 mg/l dissolved oxygen and 24±1^{o}C water temperature, in the laboratory for two week. Water was renewed daily to remove faecal matter and waste metabolite during acclimatization. Fish were fed with dried shrimp daily at particular time intervals.

2.3. Induced breeding experiment

Five female and male healthy brooders were selected for injecting different synthetic hormones (Figure 28). The hormones were injected intramuscularly near dorsal fin above lateral line with single dose of hormones viz., Ovidac: 8-17 IU/g body weight female and 3-5 IU/g body weight male, Gonopro-FH: 0.3-1.2 ml/kg body weight female and 0.1-0.2 ml/kg body weight male and Ovaprim: 0.5-1.5 ml/kg body weight female and 0.2-0.4 ml/kg body weight male). The injected brooders with different hormones kept separately in circulating water aquariums (Figure 29). The inducing hormone dose range selected as a minimum to maximum to facilitate the female stripping. After the respective latency period, the eggs were obtained by applying gentle pressure on the female abdomen. The ovulated eggs were put in the fertilization tray and further fertilized with sperm suspension which was previously prepared by mincing the testis of healthy male fish in 0.4% fish saline (Figure 30). Successful fertilization was achieved with in 30 sec. of insemination with milt or sperm suspension. The fertilized eggs were recognized with naked eye as the red cap on animal pole was oriented. Then, these eggs were transferred into rectangular hatching trays while taking precaution to avoid damage and fungal/bacterial contamination during the egg collection process. A continuous flow of water was maintained for aeration to ensure the environmental conditions were optimal for the hatching process. Fecundity was noted for each group of injected brooders. The fertilization and hatching rate was recorded for each group. The experiment was performed in triplicates. In experimental set, three number of fish was taken (n=3). The fertilization and hatching rate were calculated to know the best inducing hormone, as below:

Fertilization rate (%) = (No. of fertilized eggs/ Total no. of eggs) × 100

Hatching rate (%) = (No. of eggs hatched/ Total no. of fertilized eggs) × 100

2.4. Developmental study

The embryonic development was examined under the Bright field microscope (Olympus CX41) using micropublisher 3.3 RTV camera (Qimaging, BC, Canada) at 4X magnification. The data were expressed at mean±SEM. The one way analysis of variance (ANOVA) was used for overall significance ($p < 0.05$).

Figure 27: Sampling of experimental freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) from Gomati River, Lucknow, U.P. with the help of skilled fisherman by using drag nets.

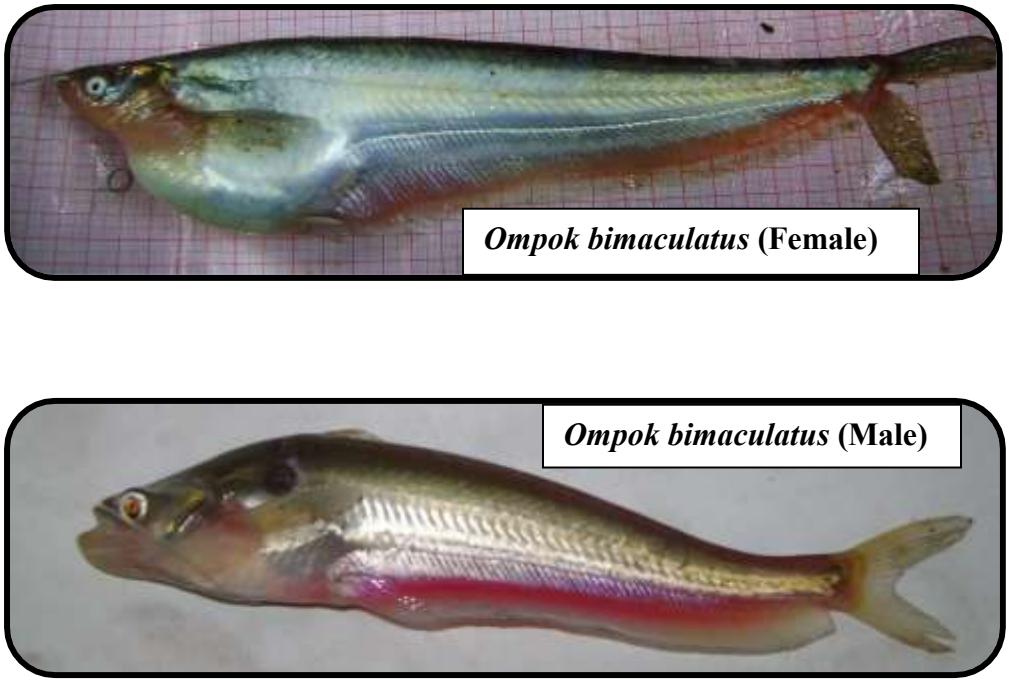

Figure 28: Healthy fresh brooders of experimental freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) sampled from Gomati River, Lucknow, U.P.

Figure 29: Injected brooders of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in well aerated glass aquaria to complete their latency period.

A

B

Figure 30:

(A) Stripping of hormone induced female brooders freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) and fertilized with milt of testis

(B) Fertilized eggs of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

3. Results

Commonly used inducing hormones (Ovidac, Gonopro-FH and Ovaprim) were tested for freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) breeding to know the best suitable inducer among tested inducers for this fish spp. The present study was resulted that all commercially available inducing hormones (Ovidac, Gonopro-FH and Ovaprim) were able to induce female fish *O. bimaculatus* ovulation with stripping in one dose only. The male and female fish were injected at the same time for induced breeding. The dosage of inducing agents was increased to know the best effective dose for induced breeding of *O. bimaculatus*. The selected range of Ovidac hormone was 8 to 17 IU/g body weight for female and 3 to 5 IU/g body weight for male fish (Figure 31). Similarly for Gonopro-FH, the range was 0.3 to 1.2 ml/kg of BW of female and 0.1 to 0.2 ml/kg of body weight of male respectively (Figure 32). In case of Ovaprim, the female fish was induced with varied range from 0.5 to 1.5 ml/kg of body weight and 0.2 to 0.4 ml/kg of body weight for male fish (Figure 33). By latency period, fecundity (number of ovulated eggs) and fertilization rate, the effective dosage of various hormones were as for Ovaprim, 1.2:0.4 ml/kg; Gonopro-FH, 0.9:0.2 ml/kg and Ovidac, 15:5 IU/g of body weight for female/male brooder in each respective group (Figure 31-33).

The current study provided a range of optimum dose of different hormones at which fish was spawned by stripping method i.e., Ovidac: 10-15 IU/g for female and 4-5 IU/g for male; Gonopro-FH: 0.5-0.9 ml/kg for female and 0.1-0.2 ml/kg for male; Ovaprim: 0.7-1.2 ml/kg for female and 0.3-0.4 ml/kg body weight male; Figure 31-33). The latency periods were different as per the inducing agents. The latency period was decreasing with increase in hormone dose in female with all three hormones. The Ovaprim injected group of fish showed 11 hr of latency period as compared to Gonopro-FH (12 hr) and Ovidac (14 hr). The number of ovulated eggs were significantly increased with increasing dose of hormones (Ovaprim: F=112.54, Gonopro-FH: F=370.29, Ovidac: F=255.5; $p < 0.05$). The maximum number of eggs were noticed in Ovaprim injected brooders (14945.88±421.5 at 1.2:0.4 ml/kg body weight: female/male) as compared to other synthetic hormones viz., Ovidac (8997.51±311.8 at 15:5 IU/kg body weight:

female/male) and Gonopro-FH (10233.32±398.7 at 0.9:0.2 ml/kg body weight: female/male) (Figure 31-33: A). The fertilization rate was significantly increased with respective increasing dose of hormones (Ovaprim: F=64.59, Gonopro-FH: F=62.74, Ovidac: F=47.45; $p < 0.05$). The fertilization rate was significantly higher in Ovaprim injected brooders i.e., 92.73±0.64% as compared to Gonopro-FH injected fish (76.41±0.87%) and Ovidac (68.38±0.51%) (F=269.79; $p < 0.05$). Later after 24 hr of fertilization, the hatching was occurred. Same pattern of increasing was recorded in hatching rate. It was also significantly increased with different doses of different hormones (Ovaprim: F=39.07, Gonopro-FH: F=31.21, Ovidac: F=15.3; $p < 0.05$). The hatching rate was ranged from 62.24±0.41% to 89±0.29% depend upon the inducing hormones (Ovaprim, Gonopro-FH and Ovidac). The Ovaprim showed significantly highest hatching rate (89±0.29%) followed by Gonopro-FH (70.76±0.35%) and comparatively less in Ovidac (62.24±0.41%) (F=99.15; $p < 0.05$) (Figure 31-34).

The embryonic development was started after eggs fertilization (Table 13). Further developmental stages were more or less similar in all tried induced breeding groups (Figure 35). The fertilized eggs were transparent with light brown in color, and unfertilized eggs were creamy white in color. The blastodisc formed after 8 min. of fertilization as a crescent- shaped light area towards the animal pole of egg (Figure 35A). The first cleavage or 2-celled stage was noticed at 30 min (Figure 35B), followed by 4- and the 8-celled stage formed at 45 min and 60 min after fertilization (Figure 35C, D). The 16-celled stage was visible at 75 min after fertilization (Figure 35E). In continuous embryonic development, the 32-celled and 64-celled stage was at 85 min and 95 min after fertilization (Figure 35F, G). The morula stage was found in 2:20 hr (Figure 35H). At the blastula stage, blastoderm cells covered one-third of the eggs space. It was seen at 3:45 h after fertilization (Figure 35I). The yolk plugged stage reached at 5:20 hr. At this stage, blastoderm cells increased rapidly in number and spread over the yolk to form this stage. At the later development, the thick portion of the embryo was formed at one end which known as the cephalic region and another thin portion was called tail region (Figure 35J). The hatching took place after 23 hr of fertilization (Figure 35K). The newly hatched larva was slender, transparent with large yolk liquefied at anterior side. At this

stage, larva had a distinct cephalic region with the brain, trunk with heart and alimentary canal and clear long tail that helps in movement (Figure 35L). Second-day larva had a clear liquefied yolk. At this stage, pigmentation was in cephalic and trunk region Larvae had a big eye and stumpy barbels, movement with the tail only (Figure 35M). Third-day larvae had the little yolk, operculum with gill, and distinguished barbels. Pigmentation increased to the whole body. Now, mouth opening and fin folds were clearly visible (Figure 35N). After three days of hatching, larva converted into fry stage (Figure 35O). Fry was an active fast swimmer and voracious eater (Figure 35O, P).

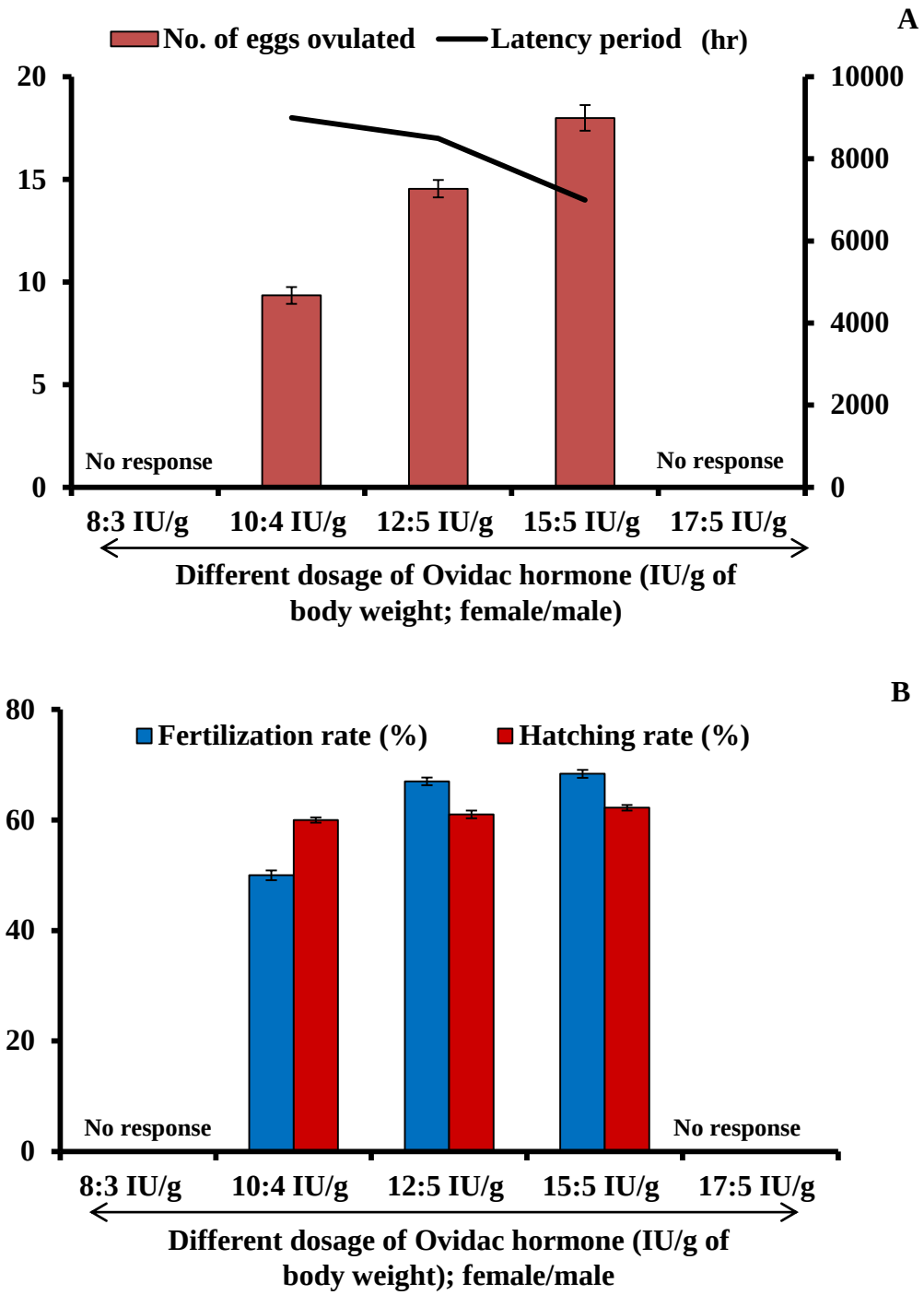

Figure 31: The latency period, number of ovulated eggs, latency period (hr), fertilization rate (%) and hatching rate (%) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) induced by different dosages of Ovidac hormone. Data were expressed in mean±SEM. Value were analysed by one way ANOVA ($p < 0.05$).

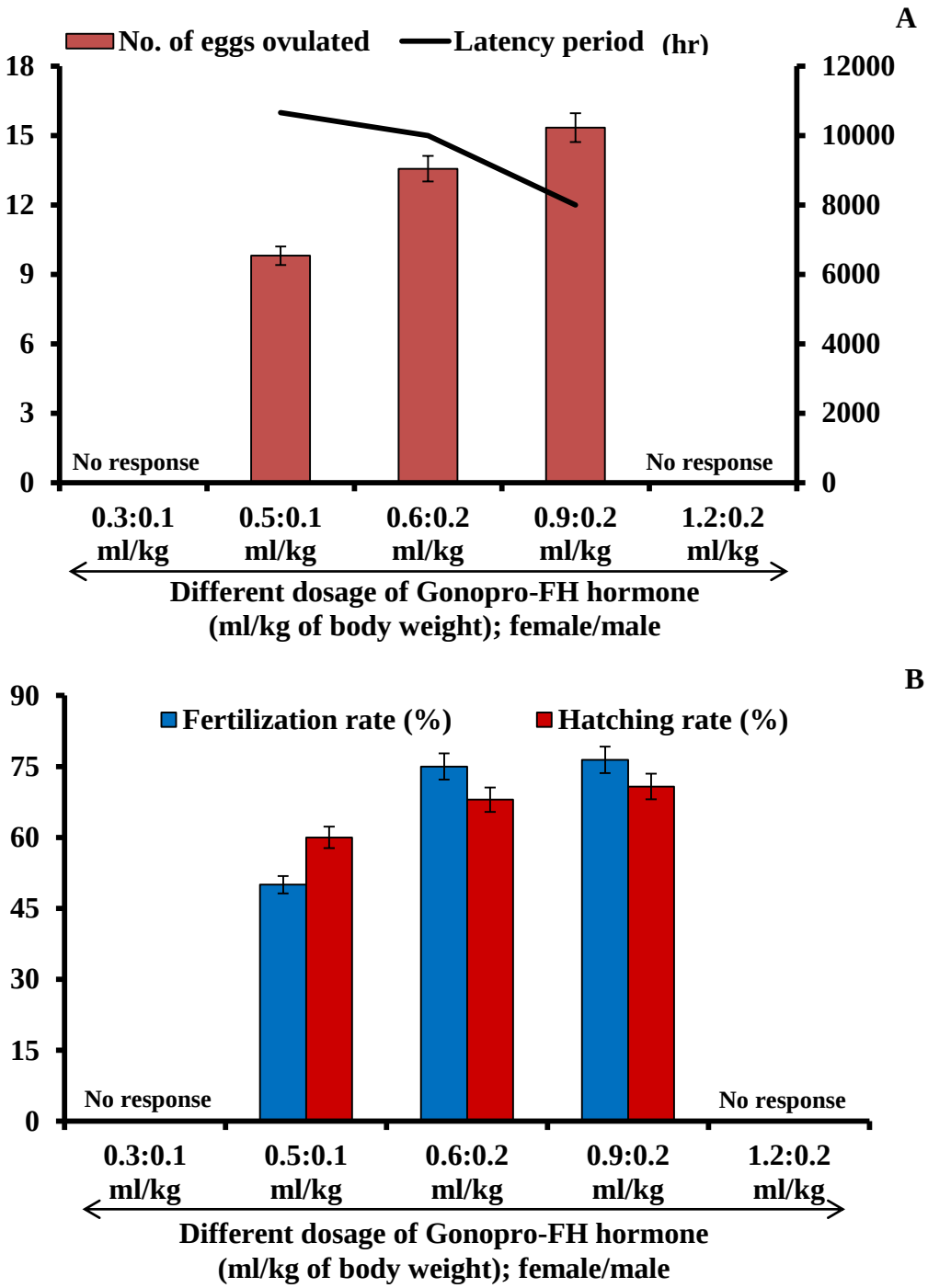

Figure 32: The latency period, number of ovulated eggs, latency period (hr), fertilization rate (%) and hatching rate (%) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) induced by different dosages of Gonopro-FH hormone. Data were expressed in mean±SEM. Value were analysed by one way ANOVA ($p < 0.05$).

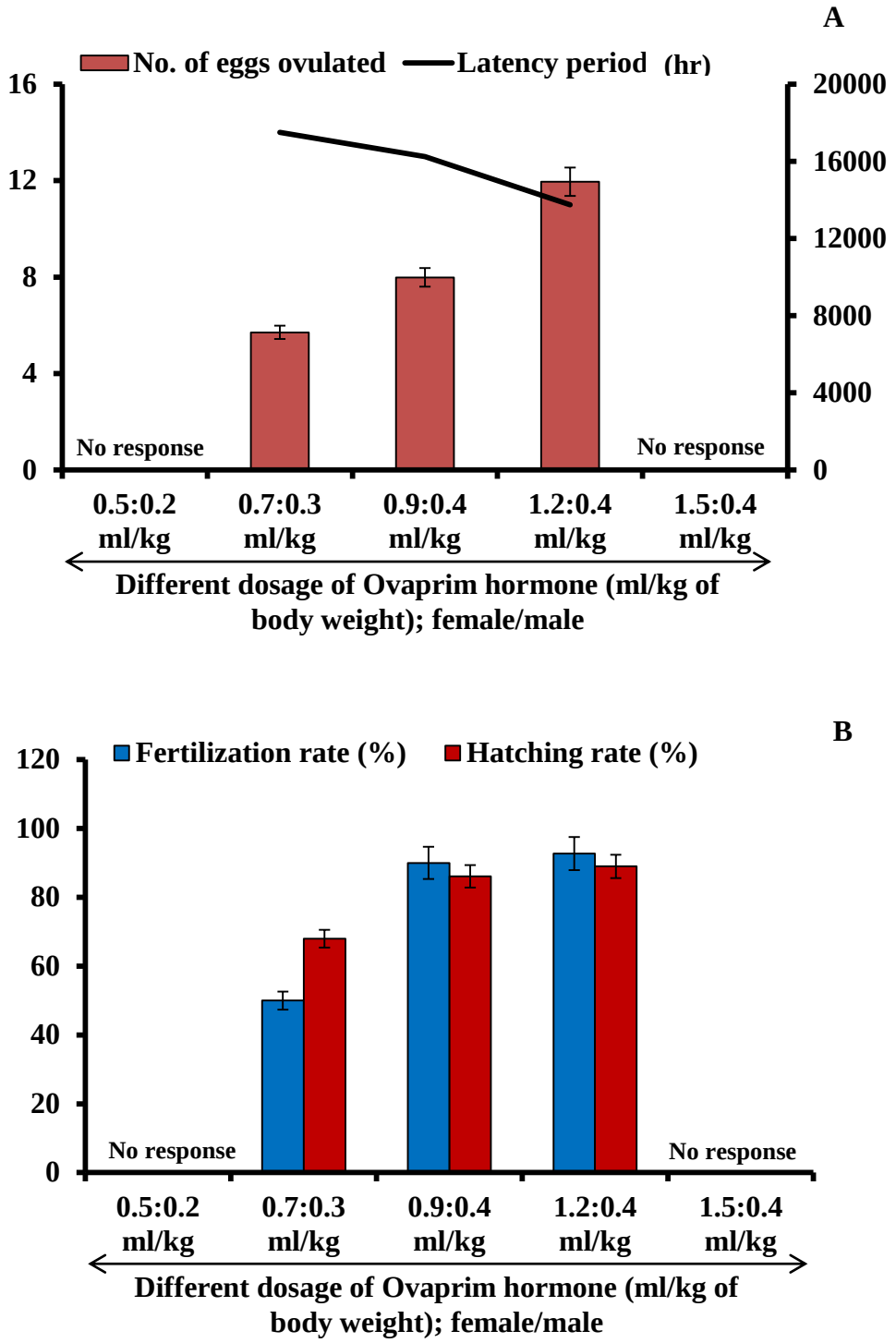

Figure 33: The latency period, number of ovulated eggs, latency period (hr), fertilization rate (%) and hatching rate (%) of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) induced by different dosages of Gonopro-FH hormone. Data were expressed in mean±SEM. Value were analysed by one way ANOVA ($p < 0.05$).

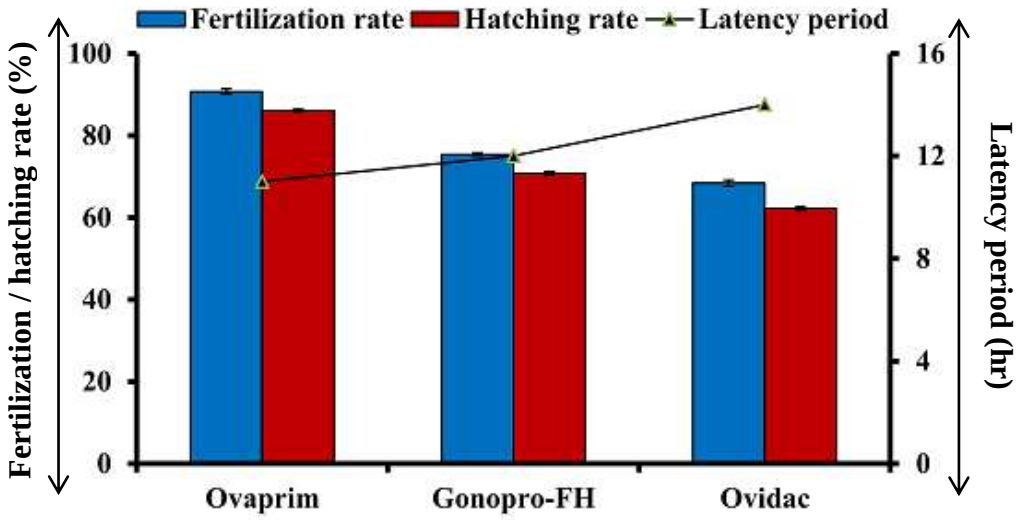

Figure 34: Fertilization rate (%), hatching rate (%) and latency period (hr) of freshwater butter catfish *Ompok bimaculatus* (bloch, 1794) induced by effective dosage of three hormones (Ovaprim: 1.2:0.4 ml/kg body weight of female/male, Gonopro-FH: 0.9:0.2 ml/kg body weight of female/male and Ovidac: 15:5 IU/g body weight of female/male). Data were expressed in mean±SEM. Value were analysed by one way ANOVA ($p < 0.05$).

Table 13: Embryonic development of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794).

S. No.	**Parameters**	**Development time (hr:min)**
1	Fertilization	0
2	Blastodisc formation	0:08
3	2 celled stage	0:30
4	4 celled stage	0:45 to 0:50
5	8 celled stage	0:60
6	16 celled stage	1:15
7	32 celled stage	1:25
8	64 celled stage	1:35
9	Morula stage	2:20
10	Blastula stage	3:45
11	Yolk plugged stage	5:20
12	Kidney shaped stage	7:40
13	Hatchling	23:00

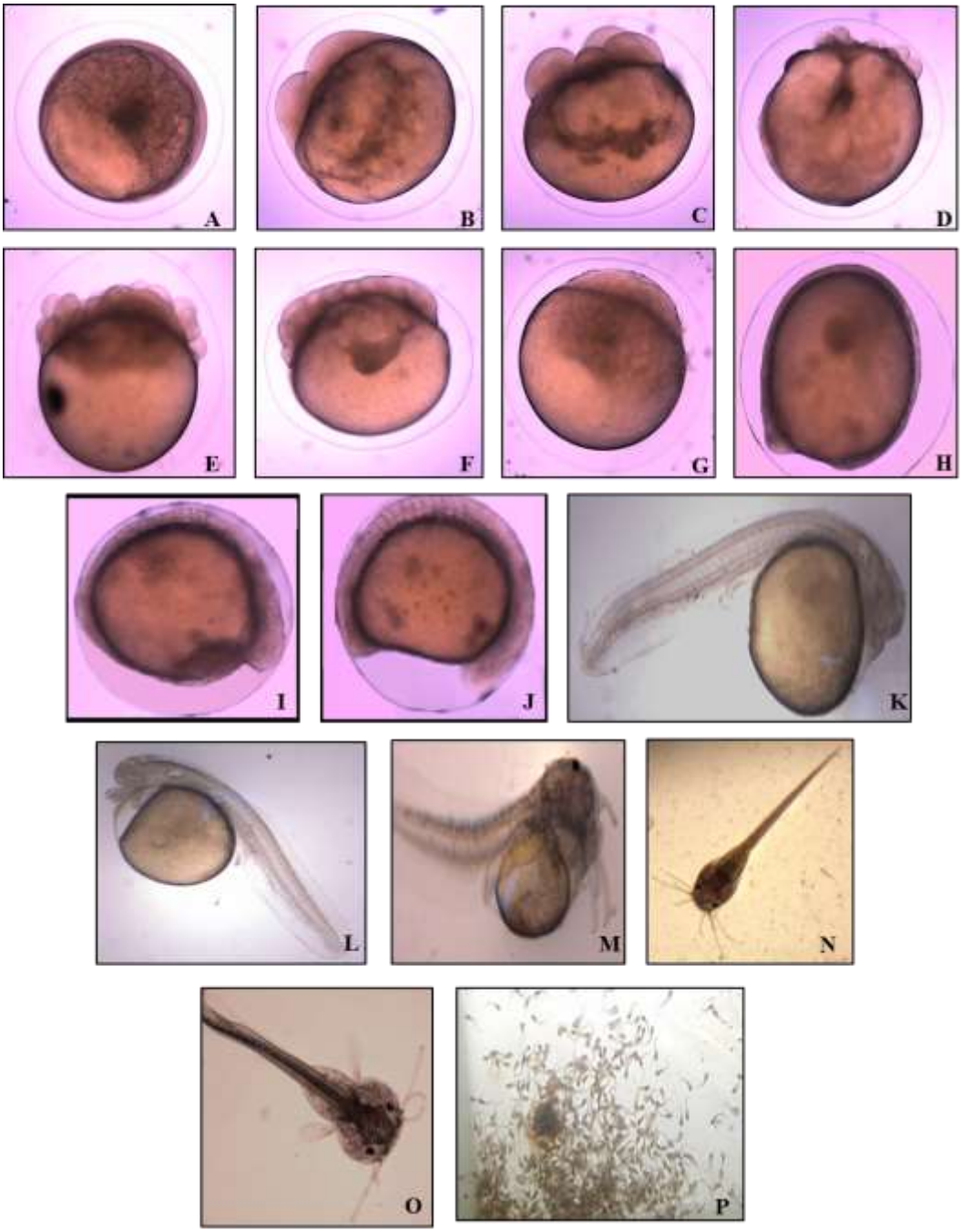

Figure 35: Developmental stages of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) showing; A: Fertilized egg, B: 2 celled stage, C: 4 celled stage, D: 8 celled stage, E: 16 celled stage, F: 32 celled stage, G: Morula stage, H: Blastula stage, I: Yolk plugged stage, J: Kidney shaped stage, K: New hatched larva, L: 1 day old hatched larva, M: 2 days old hatched larva, N: 3 days old hatched larva, O: 7 days old hatched larva, P: Hall of fry.

4. Discussion

For the better conservation of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) and an effort toward its population management strategy, induced breeding was performed with stripping method. Induced breeding is a technique whereby brooders are stimulated by using different synthetic hormones. This stimulation through hormones promotes timely release of sperms and eggs (Murthy et al., 2013). In the present study, three different hormones (Ovidac, Gonopro-FH and Ovaprim) were compared for better breeding results. These hormones were commonly available in markets. All these hormones were able to induce the experimental fish for breeding with the differences in their latency period, fecundity, fertilization rate and hatching rate. There were many studies who conducted successfully induced breeding of many fish (Nandeesha et al., 1990a, b, c; Haniffa et al., 1996; Kumar et al., 2010; Sharma et al., 2010; Singh et al., 2012; Loh and Ting, 2015).

The results showed that increasing hormone stimulus dose in male reflect with an increase in fertilization rate and the hatching rate in all groups. It supports the idea of male gamete preparation and quality improvement by the inducer hormones (Shinkafi and Ilesanmi, 2014; Yasui et al., 2015). Recently fish culturists are trying to make them breed in captivity naturally after application of spawning inducer like major carp (Sarkar et al., 2005; Ali et al., 2014; Rahman et al., 2013; Raizada et al., 2013). In the current study, inducing hormones were used in both male and female to just synchronize gamete maturation in the individual case (Targonska et al., 2011). The different combinations of inducing agents or hormones were tried. In male, the mild dose was selected as compared to female to get the best result in each group. Increase in hormone dosage caused decrease latency period, increased fecundity, and fertilization and hatching rate. Similar findings were reported by other researchers viz., Salami et al. (1994), Sarkar et al. (2005), Purkayastha et al. (2012). In higher concentration of hormone, female showed plug stage in all sets and was unable to ovulate and ultimately died. It supported that higher hormone level in fully mature fish caused severe stress and made them unable to spawn (Sahoo et al., 2008; Fernandez-Palacios et al., 2014). Sub-optimal dose (insufficient

lower hormonal dose) of inducing hormone having female were also unable to ovulate (Raizada et al., 2013; Fernandez-Palacios et al., 2014).

The latency period in the present study was 11 hr for Ovarprim, 12 hr for Gonopro-FH and 14 hr for Ovidac at their respective effective dose. The latency period was high for Ovidac hormone with the effective dose (15:5 IU/g body weight). In lower dose of hormones, the latency period was observed to more whereas in higher dosage, latency period was less. Similar observations were also reported by Habibi et al. (1989). Francis (1996) also reported high latency period for *H. fossilis* and *Clarias batrachus* due to low potency of Ovidac (Legendre, 1986). Billard et al. (1984b) and Peter et al. (1986) found that differences in dose requirement may be attributed to varied level of dopamine activity in different species of fish. *O. bimaculatus* required a threshold quantity of hormone to increase their fecundity power. This inducer value may vary according to place and fish stage (Shinkafi and Ilesanmi, 2014; Mishra et al., 2016; Sarkar et al., 2017). It supports the release of required steroid to promote oocyte maturation (MIS) and ovulation (progesterone and corticosterone) within the female body to help smooth and complete spawning (Sharaf, 2012; Fernandez-Palacios et al., 2014; Mohammadian et al., 2015).

The fertilization rate and hatching rate were also high in Ovaprim treated group of fish. Same reports of higher fertilization rate with Ovaprim treatment were observed by Nandeesha et al. (199a, b, c), Haniffa and Sridhar (2002) and More et al. (2010). The result showed that Ovaprim was the best hormone for induced breeding in freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794). The synthetic hormone, Ovaprim gave best results (Nandeesha et al., 1990a, b, c, 1993; Alok et al., 1993; Sarkar et al., 2005). It may be due to its combination that includes domperidone in addition to gonadotropin. This combination will facilitate oocyte maturation and provide ease in ovulation (Acharjee et al., 2016). Differences in the fertilization rate can be attributed to the differences in hormonal dosage, size of brooders and seasonal environmental variations (Gheyas et al., 2002; Nwokoye et al., 2007; Haniffa and Sridhar, 2002).

The embryonic development pattern of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) from fertilized ovum to fry was similar with all inducing agents as in other studies. The mild difference in duration of organogenesis may be due to the difference in the local environment (Rahman et al., 2004; Chakrabarty et al., 2008; Sarma et al., 2012a, b). Similar observation was also reported in *Ompok bimaculatus* (Bloch, 1794) (Chaudhuri, 1962; Parameswaran et al., 1967) and *Ompok pabda* by (Chakrabarty et al., 2007). Several other reports were available on various levels of success in induced breeding of many Indian fish under captivity (Ramaswamy and Sunderraj, 1969; Zairin et al., 1992; Alok et al., 1993, 1998, Sridhar et al., 1998; Bhowmik et al., 2000; Haniffa et al., 2001; Chakrabarty et al., 2006). This research will help in understanding the possibility of choosing the synthetic hormones for inducing the brooders for successful breeding and better aquaculture management for freshwater butter catfish spp. *Ompok bimaculatus.*

Consolidated Summary and conclusion

The freshwater teleost, *Ompok bimaculatus* (Bloch, 1794), is commonly known as butter catfish. It is common Asian region fish. But due to anthropogenic activities, its population is decreasing. Now this fish spp. is listed as near threatened species in IUCN Red data book. Due to its delicious taste and for sustainable catching purpose its culture practice is strongly recommended.

The present work dealt with the exploration of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) from different Indian Rivers (Betwa, Brahmaputra, Cauvery, Chambal, Ganga, Ghaghara, Godavari, Gomati, Hooghly, Krishna, Mahanadi, Narmada, Ramganga, Sharda, Sone, Subarnarekha, and Tapti). The study was focused on morpho-metric and reproductive parameters. The result suggested that Narmada River has the best sample of *Ompok bimaculatus* on body weight and depth, total length, fork length, standard length, gonadal weight, gonadosomatic index, ovarian protein and fecundity. However, oocyte weight was higher in Krishna River sample, and oocyte diameter was larger in Cauvery sample. Changes in gonadosomatic index (GSI), ovarian protein and histology observed in preparatory, pre-spawning and spawning phase of the reproductive cycle. GSI and ovarian protein concentration distributions were correlated significantly ($p < 0.05$) by linear regression analysis. The observation of fish sampled from different rivers showed that Narmada River has better adaptive condition for growth and breeding of *O. bimaculatus* in comparison of others rivers.

To promote the culture of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in North India, sampled from Gomati River, the study of its annual reproductive biology is important. Among reproductive parameters, GSI was progressively increased with the gonadal maturation. It was increased significantly in July, and reached its peak point in August month. The lowest value was recorded in November month ($p < 0.001$). The GSI data also got support from annual macroscopic and microscopic details of gonads. The GSI significantly correlated with the physico-chemical characteristics of

Gomati River water. The annual reproductive cycle of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) was described in five phases viz., resting phase (November-January), preparatory phase (February-March), pre-spawning phase (April-July), spawning phase (August) and post-spawning phase (September-October).

Induced breeding of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in Lucknow was performed by three different commercially available hormones viz., Ovaprim, Gonopro-FH and Ovidac. Both male and female brooders injected with a range of hormone in a single dose. Ovaprim has emerged best with less latency period and higher fertilization and hatching rate. The further embryonic development was alike for all tested induced steroid hormones. The result showed that near threatened freshwater butter catfish *Ompok bimaculatus* might breed in captivity like other catfish with ovaprim as a most potent exogenous hormone.

In conclusion, this attempt has been made to study the fish spawning biodiversity and to find out whether the environmental factors of rivers from different ecological regimes could be related to reproductive maturity. Despite the importance of the species of high conservation and commercial value, no published account on its spawning efficiency has been carried out with respect to the environmental factors. From the output of this research, it can be stated that the local area aquatic atmosphere plays an important role in the species specification and can affect their reproductive performance so as to their survival. This present study is necessary to a better understanding of the reproductive biology of this fish in the northern region to adopt breeding practices which is very important for sustaining the aquaculture, management and conservation of this near threatened freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in the country.

References

Abascal, F.J., Medina, A. 2005. Ultrastructure of oogenesis in the bluefin tuna, *Thunnus thynnus*. *Journal of Morphology*, 264(2): 149-160.

Abedi, M., Shiva, A.H., Mohammadi, H., Malekpour, R. 2011. Reproductive biology and age determination of *Garra rufa* Heckel, 1843 (Actinopterygii: Cyprinidae) in central Iran. *Turkish Journal of Zoology*, 35(3): 317-323.

Abell, R. 2002. Conservation biology for biodiversity crisis: a Freshwater follow-up. *Conservation Biology*, 16(5): 1435-1437.

Acharia, K., Lal. B., Singh, T.P., Pati, A.K. 2000. Circadian phase dependent thermal stimulation of ovarian recrudescence in Indian catfish, *Clarias batrachus*. *Biological Rhythm Researcch*, 31(2):125– 135.

Acharjee, A., Chaube, R., Joy, K.P. 2016. Ovaprim, a commercial spawning inducer, stimulates gonadotropin subunit gene transcriptional activity: A study correlated with plasma steroid profile, ovulation and fertilization in the catfish *Heteropneustes fossilis. General and Comparative Endocrinology*, 16 (5): 30293-3.

Adamassu, D. 1996. The breeding season of tilapia *Oreochromis niloticus* L. in Lake Awassa (Ethiopian rift valley). *Hydrobiologia*., 337: 77-93.

Adams, S.M., Bevelhimer, M.S., Greeley Jr., M.S., Levine, D.A., Teh, S.J. 1999. Ecological risk assessment in a large river-reservoir: 6. Bioindicators of fish population health. *Environmental Toxicology and Chemistry*, 18(4): 628–640.

Agostinho, C.S. 2003. Reproductive aspects of piranhas *Serrasalmus spilopleura* and *Serrasalmus marginatus* into the upper Parana River, Brazil. *Brazilian Journal of Zoology*, 63: 1–6.

Ahirrao, S.D. 2002. Status of gonads in relation to total length (TL) and gonadosomatic index (GSI) in freshwater spiny eel (pisces) *Mastacembelus armatus* (Lacepede) from Marathwada region Maharashtra. *Journal of Aquatic Biology*, 17(2): 55-57.

Ahmed, Y.A., Samei, N.A., Zayed, A.Z. 2013. Morphological and histomorphological structure of testes of the catfish *Clarias gariepinus* from Egypt. *Pakistan Journal of Biological Sciences*, 16(13): 624-629.

Akhteruzzaman, M., Kohinoor, A.H.M., Shah, M.S., Hussain, M.G. 1993. Observation on the induced breeding of silurid catfish, *Ompok pabda* (Hamilton). *Bangladesh Journal of Life Sciences*, 5(1): 71-75.

Aksnes, A., Gjerde, B., Roald, S.O. 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture*, 53 (1): 7-20.

Alam, M.D., Das, N G. 1996. Fecundity and condition index *Crassostrea maadrasensis* (Cupped oyster) in the intertidal water of the Moheshkhali channel of the Bay of Bengal. *Journal of the Inland Fisheries Society of India*, 28: 105-109.

Alam, Md., Pathak, J.K. 2010. Assessment of fecundity and gonadosomatic index of commercially important fish, *Labio rohita* from Ramganga River. *International Journal of Pharma and Biosciences*, 1: 1-6.

Ali, M.F., Rahman, M.M., Bashar, M.K., Rahmatullah, R., Hadiuzzaman, Amin, M.R., 2014. Comparative study on induced breeding of shing, *Heteropneustes fossilis* (Bloch) between HCG and PG with different combination. *International Journal of Fisheries and Aquatic Studies*, 2(2): 104-108.

Alok, D., Krishnan, T., Talwar, G.D, Garg, L.C., 1998. Multiple induced spawning of the Indian catfish *Heteropneustes fossilis* (Bloch) using D-Lys salmon gonadotropin releasing hormone analog. *Aquacuture*, 29: 252-254.

Alok, D., Krishnan, T., Talwar, G.P., Garg, L.C. 1993. Induced spawning of catfish, *Heteropneustes fossilis* (Bloch), using d-Lys^{6} salmon gonadotropin – releasing hormone analog. *Aquaculture*, 115(1-2): 159-167.

Ambily, V., Nandan, B.S. 2010. Length-weight relationship, relative condition factor (Kn) and morphometry of *Arius subrostratus* (Valenciennes, 1840) from a coastal wetland in Kerala. *Indian Journal of Fisheries*, 57(4): 39-44.

Anderson, R.O., Gutreuter, S.J. 1983. Length-weight and associated structural indices. In: fisheries techniques (Nielsen, L., Johnson, D. Ed.), American Fisheries Society, Bathesda, Maryland, pp- 283-300.

Anonymous. 2015. Annual Report 2014-15, Central Marine Fisheries Research Institute, Cochin (Kerala), India.

APHA, 2005. Standard methods for the examination of water and wastewater, 21st Ed. American Public Health Association, Washington, DC.

Arslan**,** M., Yildirim, A., Bektas, S. 2004. Length-weight relationship of brown trout, *Salmo trutta* L., inhabiting Kan stream, Coruh basin, North-Eastern Turkey. *Turkish Journal of Fisheries and Aquatic Sciences,* 4: 45-48.

Arthington, A.H., Lorenzen, K., Pusey, B.J., Abell, R., Halls, A., Winemiller, K.O., Arrington, D.A., Baran, E. 2004. River fisheries: ecological basis for management and conservation. In: Welcomme, R., Peter, T. (eds) Proceedings of the second international symposium on the management of large rivers for fisheries volume I. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand. RAP Publication, pp 21–60.

Arthington, A.H., Welcomme, R.L. 1995. The condition of large river systems of the world. In: Voigtlander, C.W. (ed) Proceedings of the world fisheries congress, chapter 4. Oxford and IBH Publishing Co Pty Ltd., New Delhi, pp 44–75.

Atkore, V.M., Sivakumar, K., Johnsingh, A.J.T. 2011. Patterns of diversity and conservation status of freshwater fishes in the tributaries of river Ramganga in the shiwaliks of the western Himalaya. *Current Science,* 100(5): 731-736.

Ayyappan, S., Biradar, R.S. 2004. Fisheries Education in India. *Fishing chimes*, 22 (1): 45-46.

Azevedo, M.A. 2000. Biologia reprodutiva de dois glandulocaudíneos com inseminacao, *Mimagoniates microlepise, Mimagoniates rheocharis* (Teleostei: Characidae), characteristics de seus ambientes. Unpublished M.Sc. Dissertation, Universidade Federal do Rio Grande do Sul, Porto Alegre, 84p.

Babiker, M.M., Ibrahim, H. 1979. Studies on the biology of reproduction in the cichlid *Tilapia nilotica* (L): gonadal maturation and fecundity. *Journal of Fish Biology,* 14(5): 437-448.

Bagarinao, T., Chua, T.E., Dizon, L.B. 1986. Egg size and larval size among teleosts: Implications to survival potential. Maclean, J.L., Hosillos, LV. (eds.), In: Proceedings of the first Asian fisheries forum, Manila, Philippines, pp 651-656.

Bagenal, T.B. 1978a. Methods for assessment of fish production in freshwater. 3rd edn., Blackwell Scientific Publication, London, ISBN: 9780632001255, pp 365.

Bagenal, T.B. 1978b. Aspects of fish fecundity. In: Shelby, D.G., ed. Ecology of fresh water fish population. 2nd ed. London, Black Well, pp 101-135.

Bagenal, T.B., Braum, E. 1978. Eggs and early life history. In: Methods of assessment of fish production in fresh waters. IBP Handbook 3. Bagenal, T.B., (Ed.). Blackwell Scientific, Oxford, pp 165–201.

Bagenal, T.B., Tesch, F.W. 1978. Methods for assessment of fish production in freshwater. Blackwell Scientific Publication, Oxford, UK., pp 101-136.

Banik, S., Bhattacharya, P. 2012. *Ompok pabo* (Hamilton, 1822) of Tripura, India: An endangered fish species in relation to some biological parameters. *Research Journal of Biology*, 2(3): 91-97.

Banik, S., Goswami, P., Acharjee, T., Malla, S. 2012. *Ompok pabda* (Hamilton-Buchanan, 1822): An endangered catfish of Tripura, India: Reproductive physiology related to freshwater lotic environment. *Journal of Environment*, 1: 45-55.

Banik, S., Goswami, P., Malla, S. 2011. Ex-situ studies of captive breeding of *Ompok bimaculatus* (Bloch, 1794) in Tripura. *Journal of Advance Laboratory Research Biology*, 2: 133-137.

Banik, S., Malla, S. 2009. Rotifer as growth promoter for a rare fish *Ompok bimaculatus* related to climate change. Proceedings of the workshop on aquaculture biotechnology for rural development, West Tripura India, Dept. of Zoology, Tripura University, pp 18-28.

Banik, S., Malla, S. 2014. Survival and growth rate of the Larvae of *Ompok pabda* (Hamilton-Buchanan, 1822) of Tripura, India: related to efficient feed. *Proceedings of Zoological Society*, 68(2): 164-171.

Banik, S., Som, S., Roy, N., Nath, S., Choudhary, S. 2002. Threatened fish fauna of Tripura: Stratagies for conservation. In: Banik, S. ed. Proceedings of national seminar on conservation of aquaculture resources, Department of Life Science, Tripura University, India. Tripura, pp 62-67.

Bano, Y. 1977. Seasonal variation in the biochemical composition of *Clarius batrachus*. L. *Proceedings of Indian Academy of Sciences,* 85 (3): 147- 155.

Barman, R.P. 1994. Fish fauna of Tripura, North-East India. *Journal of Bombay Natural History Society,* 91: 37-46.

Barmanh, M.R., Saikia, S.J. 1995. Length-weight relationship and some ecological observations of *Cirrhina reba* (ham) from wet lands of Assam, India. *Environment and Ecology,* 13(3): 721-724

Bayley, P.B. 1991. Personal communication to Paul Seelbach with unpublished length-weight equations for many fish species in Illinois waters.

Begum, A., Harikrishna. 2008. Study on the quality of water in some streams of Cauvery river. *E- Journal of Chemistry*, 5(2): 377-384.

Begum, K. 1997. A study on the histological analysis of gonad development, fecundity, embryonic and larval development of *Ompok pabda* (Hamilton Buchanan 1822). M.Sc. Dissertation, Department of Zoology, Dhaka University, Dhaka, Bangladesh, pp 108.

Belsare, D.K. 1962. Seasonal changes in the ovary of *Ophiocephalus punctatus*. *Indian Journal of Fisheries*, 9: 140-156.

Bhowmik, M.L., Mondal, S.C., Chakrabarti, P.P., Das, N.K., Das, K.M., Saha, R.N., Ayyappan, S. 2000. Captive breeding and rearing of *Ompok pabo* (Hamilton-Buchanan) a threatened species, pp 120-121. In Ponniah, A.G., Sarkar, U.K. (eds). Fish biodiversity of North East India. NBFGR. NATP Publ. 2, pp 228.

Bijukumar, A. 2000. Exotic fishes and freshwater fish diversity. *Zoos' Print J.,* 15(11): 363-367.

Bilgrami, K.S., Munshi, D.J.S. 1985. Ecology of River Ganges (Patna-Farakka); impact of human activities and conservation of aquatic biota. Final technical report, Department of Environment Research Project under MAB Programme, India.

Billard, R., Bieniarz, K., Peter, R.E., Sokolowska, M., Weil, C., Crim, L.W. 1984a. Effects of LHRH and LHRH-a on plasma GtH levels and maturation/ ovulation in common carp, *Cyprinus carpio* kept under various environmental conditions. *Aquaculture*, 41: 245-54.

Billard, R., Reinaud, P., Hollebecq, M.G., Breton, B. 1984b. Advancement and synchronization of spawning in *Salmo gairdneri* and *S. trutta* following administration of LHRHa – combined or not with pimozide. *Aquaculture*, 43: 57-66.

Biswas, S.P., Borbora, S.H., Dutta, B.C. 1995. Present status of capture fisheries in the upper stretches of the Brahmaputra. *Bulletin of Life Science*, 5: 31–40.

Biswas, S.P., Boruah, S. 2000a. Ecology of the River Dolphin (*Platanista gangetica*) in the upper Brahmaputra. *Hydrobiologia*, 430: 97–111.

Biswas, S.P., Boruah, S. 2000b. Fisheries ecology of the North-Eastern himalayas with special reference to the Brahmaputra River. *Ecological Engineering*, 16: 39–50.

Biswas, S.P., Michael, R.G. 1992. Fishery characteristics and the present status of fisheries of the River Brahmaputra. Proc. seminar on conservation of river Dolphin in Indian Sub-continent, New Delhi.

Blay Jr, J., Eyeson, K.N. 1982. Observations on the reproductive biology of the shad *Ethmalosa fimbriata* (Browdich), in the coastal waters of cape coast Ghana. *Journal of Fish Biology*, 21: 485-496.

Boy, C.C., Perez, A.F., Lattuca, M.E., Calvo, J., Morriconi, E. 2009. Reproductive biology of *Galaxias maculatus* (Jenyns 1842) in the Rio Ovando, a high-latitude environment in southernmost Patagonia, Argentina. *Journal of Applied Ichthyology*, 25: 661–668.

Brinsmead J., Fox M.G. 2002. Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. *Journal of Fish Biology*, 61: 1619–1638.

Brown-Peterson, N.J., Peterson, M.S., Nieland, D.L., Murphy, M.D., Taylor, R.G., Warren, J.R. 2002. Reproductive biology of female spotted sea trout, *Cynoscion nebulosus*, in the Gulf of Mexico: differences among estuaries? *Environmental biology of Fishes*, 63: 405–415.

Burton, M.P., Idler, D.R. 1984. The reproductive cycle in winter flounder, *Pseudopleuronectes americanus* (Walbaum). *Canadian Journal of Zoology*, 62(2): 2563-2567.

Bye, V.1. 1990. Temperate marine teleosts. In: Reproductive seasonality, in teleosts. Environmental influences. Munro., A.D., Scott, A.P., Lam, T.J. (eds.) CRC. Press, Inc., Boca Raton, Florida, pp 125-143.

Cadrin, S.X., Friedland, K.D. 1999. The utility of image processing techniques for morphometric analysis and stock identification. *Fisheries Research*, 43: 129-139.

CAMP: Report of the workshop on conservation assessment and management plan (CAMP) for freshwater fishes of India, Zoo Outreach organization and NBFGR, Lucknow, India. 156.1998

Cavalcanti, M.J., Monteiro, L.R., Lopes, P.R.D. 1999. Landmark based morphometric analysis in selected species of Serranid fishes (Perciformes: Teleostei). *Zoological Studies*, 38(3): 287-294.

Cek, S., Bromage, N., Randall, C., Rana, K. 2001. Oogenesis, hepatosomatic and gonadosomatic indexes and sex ratio in roxy barb (*Punctius conchonius*). *Turkish Journal of Fisheries and Aquatic Science*, 1: 33-41.

Cek, S., Yilmaz, E. 2007. Gonad development and sex ratio of sharptooth catfish (*Clarias gariepinus* Burchell, 1822) cultured under laboratory conditions. *Turkish Journal of Zoology*, 1: 35-46.

Chakrabarti, N.M., Chakrabarti. P.P., Mondal, S.C. 2009. *Ompok bimaculatus* and *Ompok pabda*: Comparative morphometric and meristic study of embryonic larval development. *Fishing Chimes*, 29(6): 8-9.

Chakrabarti, P., Bose, S. 2014. Cyclical rhythms in the cytomorphology of testis of brackish water grey mullet *Liza parsia* (Hamilton, 1822) inhabiting south-eastern coast of India. *Journal of Entomology and Zoology Studies*, 2: 110-118.

Chakrabarti, P., Gupta, A.K. 1994. A histological study of the testes during growth, maturation and spawning phase in the exotic fish *Puntius javanicus* (Blkr.) *Advances in Biosciences*, 13: 11-12.

Chakrabarty, N.M., Chakrabarthy, P.P., Mondal, S.C. 2006. Mass seed production of pabda and their farming- a challenging step for sustainable utilization of vulnerable fish species. *Fishin Chimes*, 26(1):135-137.

Chakrabarty, N.M., Chakrabarty, P.P, Mondal, S.C., Sarangi, N. 2008. Embryonic development of Pabda (*Ompak pabda*) with notes on its farming. *Fishing Chimes,* 28 (1): 55-59.

Chakrabarty, N.M., Chakrabarty, P.P. 2005. Embryonic development and larval rearing of *Ompok pabda* (Hamilton). *Journal of the Inland Fisheries Society of India*, 37(1): 71-74.

Chakrabarty, N.M., Mondal, S.C., Chakrabarty, P.P. 2007. Artificial breeding and rearing of butter fish *Ompok pabda*- a significant mile stone in technology advancement. *Fishing Chimes*, 6(10): 134-136.

Chakraborty, B.K., Mirza, Z.A., Chakraborty, A. 2010. Reproductive cycle of the endangered pabda, *Ompok pabda* (Hamilton-Bouchanan, 1822) in Bangladesh. *Asian Fisheries Society*, 23: 301-320.

Chakraborty, B.K., Mirza, Z.A., Miah, M.I., Habib, M.A.B., Chakraborty, A. 2007. Reproductive cycle of the endangered sarpunti, *Puntius sarana* (Hamilton, 1822) in Bangladesh. *Asian Fisheries Society,* 20: 145-164.

Chakraborty, P., Bhattacharya, S. 1984. Plasma thyroxine levels in freshwater perch: Influence of season, gonadotropins and gonadal hormones. *Genernal and Comparative Endocrinology,* 53: 179-186.

Chandrasekhara, R.A., Krishnan, L. 2011. Biochemical composition and changes in biological indices associated with maturation of ovary in the spiny cheek grouper, *Epinephelus diacanthus* (Valenciennes, 1828). *Indian Journal of Fisheries,* 58(2): 45-52.

Chaudhuri, H. 1962. Induced breeding and development of a common catfish, *Ompok bimaculatus* (Bloch.). *Proceedings of Indian Science Congress,* 49: 390-391.

Chaves-Pozo, E.V., Mulero, J., Meseguer, Ayala, A.G., 2005. An overview of cell renewal in the testis throughout the reproductive cycle of a seasonal breeding teleost, the gilthead seabream (*Sparus aurata* L.). *Biology of Reproduction,* 72: 593-601.

Cheah, S.H., Yeo, M.E. 1994. Induced spawning of the catfish *Clarias batrachus* (Linnaeus) with Ovaprim. In: The Third Asian Fisheries Forum (Eds. Chuo, L.M.,

Munro A.D., Lam.T.J., Chen, T.W., Cheong, L.K.K., Ding, J.K. Hooi, K.K.Khoo, H.W., Phang, V.P.E. Shim K.F., Tan, C.H.), pp 826-829.

Chech, Jr.J.J., Moyle, P.B. 2004. Reproduction. In: Fishes-An introduction to Ichthyology. Upper Saddle River, Prentice Hall, pp 726.

Chelemal, M., Jamili, S., Sharifpour, I. 2009. Reproductive biology and histological studies in abu mullet *Liza abu* in the water of Khozestan province. *Journal of Fisheries and Aquatic Sciecne,* 4: 1-11.

Chellappa, S., Lima, J.T., Araujo, A., Chellappa, N.T. 2010. Ovarian development and spawning of *Sera Spanish mackerel* in coastal waters of northern Brazil. *Brazilian Journal of Biology*, 70: 451-456.

Chen, K.S., Crone, P.R., Hsu, C.C. 2010. Reproductive biology of albacore *Thunnus alalunga*. *Journal of Fish Biology,* 77: 119-136.

Choudhury, H. 1962. Induced breeding and development of a common catfish, *Ompok bimaculatus* (Bloch). *Proceedings of Indian Science Congress,* 49(3): 390-391.

Choudhury, M., Chandra, R., Kolekar, V. 1990. Observations on some biological and fishery aspects of *Hilisa ilisha* (Ham) of river Brahmaputra. *Journal of the Inland Fisheries Society of India*, 22: 66-74

Chovance, A., Hoffer, R., Schiemer, F. 2003. Fish as bioindicators. In: Market, B.A., Breure, A.M., Zechmeister, H.G. (eds). *Bioindicatos and Biomonitors*, 639–675

Clark, F.N. 1934. Maturity of the California sardine *Sardina caerulea* determined by ova diameter measurements. Calif. Dep. Fish game. *Fish Sou*, 42: 1-49.

Coward, K., Bromage, N.R. 1998. Histological classification of ovarian growth and the dynamics of ovarian recrudescence in *Tilapia zillii* (Gervais). *Journal of Fish Biology*, 53: 285-302.

Crim, L.W. 1991. Hormonal manipulation of fish reproductive cycles. In: Proc. 4th Int. Symp. Reproductive Physiology, of Fish. Scott, A.P., Sumpter, J.P., Kime, D.E., Rolfe, M.S. (Eds). Noewich, U.K. pp 43-47.

Cyrus, D.P., Blaber, S.I.M. 1984. The reproductive biology of Gerres in Natal estuaries. Fish Biology, 24: 491-503.

Dadzie, S.F., Abou-Seedo, Al-Shallal, T. 2000. Reproductive biology of the silver Pomfret *Pampus argentens* (Euphrasen) in Kuwail water. *Journal of Applied Ichthyology,* 16: 247-253

Darwall, W.R.T., Vie, J.C. 2005. Identifying important sites for conservation of freshwater biodiversity: extending the species based approach. *Fisheries Managemnet and Ecology,* 12: 287–293.

Das, S.K. 2002. Seed production of Magur (*Clarias batrachus*) using a rural model portable hatchery in Assam, India-A farmer proven technology. *Aquaculture Asia,* 7(2): 19-20.

Das, S.K., Kalita, N. 2003. Captive breeding of Peacock eel, *Macrognathus aculeatus. Aquaculture Asia.,* 8 (3): 17-21.

Datta, S., Rahman, M.M., Haque, M.A., Islam, M.M., Mollah, M.F.A. 2003. Chromosomal studies on *Ompok pabda* (Hamilton). *On Line Journal of Biological Sciences*., 3(5): 460-465.

Dawson, T.P., Berry, P.M., Kampa, E. 2003. Climate change impacts on freshwater wetland habitat. *Journal of Nature Conservation*, 11: 25–30.

Day, F. 1875-1878. The fishes of India: being a natural history of the fishes known to inhabit the seas and freshwaters of India, Burma and Ceylon. Text and atlas in 4 parts. London, pp 178: 195.

De Carvalho, P.A., Paschoalini, A.L., Santos, G.B, Rizzo, E., Bazzoli, N. 2009. Reproductive biology of *Astyanax fasciatus* (Pisces: Characiformes) in a reservoir in Southeastern Brazil. *Journal of Appied Ichthyology*, 25: 306-313.

De Leeuw, R., Goos, H.J.T., Richter, C.J.J., Eding, E.H. (1985). Pimozide-LHRHa induced breeding in the African catfish, *Clarius gariepinus* (Burchell). *Aquaculture,* 44: 295–302.

De Silva, S.S., Abery, N.W. 2007. Endemic freshwater finfish of Asia: distribution and conservation status. *Diversity and DistributionJournal*, 13: 172–174

de Vlaming, V.L. 1975. Effects of photoperiod and temperature on gonadal activity in the cyprinid teleost *Notemigonus crysoleucas*. *The Biology Bulletin*, 148: 402 – 415.

Deepak, P.K., Sarkar, U.K., Negi, R.S. 2008. Age and growth profile of Indian major carp *Catla catla* from rivers of Northern India. *Acta Zoologica Sinica,* 54: 136-143.

Delahunty, G., de Vlaming, V.L. 1980. Seasonal relationship of ovary weight, liver weight and fat stores with body weight in the gold fish, *Carassius auratus* (L.). *Journal of Fish Biology,* 16 (1): 5-13.

DeMartini, E.E., Lau, B.B. 1999. Morphometric criteria for estimating sexual matuarity in two snappers, *Etelis carbunculus* and *Pristipomoides sieboldii*. *Journal of Fish Biology,* 97: 449-458.

Depledge, M.H., Fossi, M.C. 1994. The role of biomarkers in environmental assessment (2). Invertebrates. *Ecotoxicology*, 3: 161–172.

Devaraj, M. 1973. Biology of the large snake-head *Ophioeephalus marulius* (Ham.) in Bhavani sagar waters. *Indian Journal of Fisheries,* 20: 280-303.

Dewan, S., Doha, S. 1979. Spawning and fecundity of certain pond fishes. *Bangladesh Journal of Agriculture,* 4(1): 1-8.

Doha, S., Hye, M.A. 1970. Fecundity of padma River hilsa, *Hilsa ilisha* (Ham.). *Pakistan Journal of Science*, 22: 176-184.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81:163–182

Dynes, J., Magnan, P., Bernatchez, L., Rodríguez, M.A. 1999. Genetic and morphological variation between two forms of lacustrine brook charr. *Journal of Fish Biology,* 54: 955-972.

Dziewulska, K., Domagala, J. 2003. Histology of salmonid testes during maturation. *Reproductive Biology,* 3: 47-61.

Ebisawa, A. 1990. Reproductive biology of *Lethrinus nebulosus* (Pisces: Lethrinidae) Around the Okinawan Waters. *Nippon Suisan Gakkaishi,* 56(12): 1941-1954.

El-Gohary, N.M.A. 2001. The effect of water quality on the reproductive biology of the Nile tilapia, Oreochromis niloticus in Lake Manzalah. Ph.D. Thesis. Faculty of Science, Ain Shams University.

El-Sayed, A.M., Mansour, C.R., Ezzat, A.A. 2003. Effects of dietary protein level on spawing performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. *Aquaculture,* 220: 619-632.

Encina, L., Lorencio, C.G. 1997. Seasonal changes in condition, nutrition, gonad maturation and energy content in barbel, *Barbus sclateri* inhabiting a fluctuating river. *Environmental Biology of Fishes,* 50(1): 75-84.

Estay, F., Neira, R., Diaz, N.F., Valladares, L., Torres, A. 1998. Gametogenesis and sex steroid profiles in cultured coho salmon (*Oncorhynchus kisutch*, Walbaum). *Journal of Experimental Zoology*, 280: 429-438.

Etienne, V. deV. 1982. Fecundity. Multilingual demographic dictionary, English section, second edition. Demopaedia. org, International Union for the Scientific Study of Population. Edited by Louis, H., pp 621-1.

Eugene, G. 1958. "Fecundity". Multilingual demographic dictionary, English section. Prepared by the Demographic Dictionary Committee of the International Union for the Scientific Study of Population. Demopaedia.org, United Nations Department of Economic and Social Affairs (DESA), pp. 621-1.

Fawole, O.O., Arawomo, G.A.O. 2000. Fecundity of *Sarotherodon galilaeus* (Pisces: Cichlidae) in the Opa reservoir, Ile-Ife, Nigeria. *Revista de Biologia Tropical*, 48: 201–204.

Fermin, J.D.T. 1992. Induction of oocyte maturation and ovulation in the freshwater Asian catfish *Clarius macrocephalus* by LHRHa and pimozide. *Journal of Applied Ichthylogy*, 8: 90-98.

Fernandez-Palacios, H., Schuchardt, D., Roo, J., Izquierdo, M., Hernandez-Cruz, C., Duncan. N. 2014. Dose-dependent effect of a single GnRHa injection on the spawning of meagre (*Argyrosomus regius*) broodstock reared in captivity. *Spanish Journal of Agricultural Research*, 12(4): 1038-1048.

Fishelson, L., Goren, M., Van, V.J., Manelis, R. 1996. Some aspects of the reproductive biology of Barbus spp., *Capoeta damascina* and their hybrids (Cyprinidae, Teleostei) in Israel. *Hydrobiologia*, 317: 79-88.

Fontoura, N.F., Braun, A.S., Milani P.C.C. 2009. Estimating size at first maturity (L) from gonadosomatic index (GSI) data. *Neotropical Ichthyology*, 7: 217-222.

Foucher, R.P., Beamish, R.J. 1980. Production of noviable oocytes by pacific hake (*Merluccius productus*). *Canadian Journal of Fish and Aquatic Science*, 37: 41-48.

Francis, T. 1996. Studies on the effect of pituitary hormone and feeds on the reproduction of Heteropneustes fossilis (Bloch). PhD Thesis. Tamil Nadu Veterinary and Animal Science University, Madras, India.

Froese, R. 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. *Journal ofApplied Ichthyology*, 22: 241–253.

Furbock, S., Patzner, R.A., Lahnsteiner, F. 2010. Fine structure of spermatozoa of *Chondrostoma nasus* and *Rutilus meidingerii* (Teleostei, Cyprinidae), as revealed by scanning and transmission electron microscopy. *Acta zoologica,* 91: 88-95.

Garg, S.K., Jain, S.K. 1984. Effect of photoperiod and temperature on ovarian activity in the Indian murrel, *Channa* (Ophiocephalus) *punctatus* (Bloch). *Canadian Journal of Zoology*, 63: 834-842.

Garg, S.K., Sundararaj, B.I. 1985. Response of the testes and seminal vesicle of the catfish, *Heteropneustes fossilis* (Bloch) to various combinations of photoperiod and temperature. *Physiological Zoology*, 58: 616- 627

Gentile, F., Lira, O., Marcano, D. 1986. Relationship between brain gonadotrophin-releasing hormone (GnRH) and seasonal reproductive cycle of ‘‘*Caribe Colorado*’’, *Pygocentrus notatus. General and Comparative Endocrinology*, 64: 239–245.

Georgakopoulou, E., Sfakianakis, D.G., Kouttouki, S., Divanach, P., Kentouri, M., Koumoundouros, G. 2007. The influence of temperature during early life on phenotypic expression at later ontogenetic stages in sea bass. *Journal of Fish biology*, 70: 278-291.

Ghanbahadur, A.G., Ghanbahadur, G.R. 2012. Study of gonadosomatic index of fresh water fish, *Cyprinus carpio*. *Trends in Fisheries Research*, 1: 32-33.

Gheyas, A.A., Islam, M.S., Mollah, M.F.A., Hussain, M.G. 2002. A comparative study on the embryonic development of gynogen, triploid, haploid and normal diploid

of stinging catfish, *Heteropneustes fossilis*. *Bangladesh Journal of Fisheries Research* 6(2): 107-115.

Gibbs, J.P. 2000. Wetland loss and biodiversity conservation. *Conservative Biology*, 14(1), 314–317.

Golterman, H.L., Cyclo, R.S., Ohanstad, M.A.M. 1978. Method for physical and chemical analysis of fresh water. IBP handbook No 8. Blackwell Sci. Pub., Oxford, UK.

Gomes, L.D., Araujo, F.G., Uehara, W., Sales, A. 2011. Reproductive biology of the armoured catfish *Loricariichthys castaneus* (Castelnau, 1855) in Lajes reservoir, southeastern Brazil. *Journal of Applied Ichthyology*, 27: 1322-1331.

Gonzalez, A.A.F., De La Cruz Aguero, G., De La Cruz Aguero, J. 2004. Length-weight relationships of fish caught in a mangrove swamp in the Gulf of California (Mexico). *Journal of Applied Ichthyology*, 20: 154-155.

Gonzalez, J.A. 1980. Reproduccio´n y crecimiento del "*Caribe Colorado" Serrasalmus notatus* Lutken, 1974 (Teleostei, Characiformes, Characidae) en los Llanos Venezolanos. Trabajo Especial de Grado. Escuela de Biologı´a. Facultad de Ciencias. UCV. Caracas, Venezuela.

Goos, H.J.Th., Joy, K.P., de Leeuw, R., Van Oordt, P.G.W.J., Van Delft, A.L.M., Gielen, J.Th. 1987. The effect of lutenizing hormone-releasing hormone analogue (LHRHa) in combination with different drugs with anti-dopamine and anti-serotonin properties on gonadotropin release and ovulation in the African catfish, Clarias gariepinus. *Aquaculture*, 63: 143–156

Goswami, U.C., Sarma, N.N. 1997. Pituitary dose optimization for induced ovulation, in vitro fertilization and production of normal fry of *Clarias batrachus* (Linn.). *Asian Fisheries Society*, 10: 163-167.

Grau, A., Linde, M., Grau, A.M. 2009. Reproductive biology of the vulnerable species *Sciaena umbra* Linnaeus, 1758 (Pisces: Sciaenidae). *Scientia Marina*, 73: 67- 81.

Guerrero, H.Y., Caceres, G., Paiva, C.L., Marcano, D. 1990. Hypothalamic and telencephalic catecholamine content in the brain of the teleost fish, *Pygocentrus notatus*, during the annual reproductive cycle. *General and Comparative Endocrinology*, 80: 257–263.

Gupta, J.P., Shrivastava, S.P. 2001. Maturity determination by gonadosomatic index during annual reproductive cycle in *Passer domesticus*. *Environment and Ecology*, 191: 216-218.

Gupta, S., Banerjee, S. 2013. Studies on some aspects of Reproductive biology of *Amblypharyngodon mola* (Hamilton-Buchanan, 1822). *International Research Journal of Biological Science*s, 2(2): 69-77.

Guraya, S.S. 1986. The cell and Molecular Biology of Fish Oogenesis; Monographs in development biology. S Karger (Publisher), 18: 223.

Guraya, S.S. 1993. Follicular atresia and its causes in the fish ovary. *Adv. Fish Res.*, 1: 313-332.

Guraya, S.S. 2000. The biology of gonadal development, sex differentiation, maturation and sex reversal in fish; cellular, molecular and endocrinological aspects. *Proceedings of the National Academy of Sciences, India*, 4-5: 167-194.

Habibi, H.R., Marchant, T.A., Nahorniak, C.S., Vanderloo, H., Peter, R.E., Rivier, J.E., Vale, W.W. 1989. Functional relationship between receptopuir binding and biological activity for analogous of mammalian and salmon gonadotropin releasing hormone in the pituitary of Goldfish (*Carrassius auratus*). *Biology Repots*, 40: 1152-1161.

Ham, K.D., Adams, S.M., Peterson, M.J. 1997. Application of multiple bio-indicators to differentiate spatial and temporal variability from the effects of contaminant exposure on fish. *Ecotoxicology and Environmental Safety*, 37: 53–61.

Hamilton, F. 1822. An account of the fishes found in the river Ganges and its branches. Edinburg and London, VIII: 405.

Haniffa, M.A., Merlin, R.T., Shaik, M.J. 1996. Induced of ovulation in Channa striatus by salmon gonadotropin releasing hormone analogue (GnRH). *Fishing Chimes*, 16: 23-24.

Haniffa, M.A.K., Sridhar, S. 2002. Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionic gonadotropin and synthetic hormone (Ovaprim). *Veterinarski Archiv*, 72: 51-56.

Haniffa,M.A., Jesu, A.R.A., Verma, T.M.A. 2001. Optimum rearing conditions for successful artificial propagation of catfish. In Ponniah, A.G., Lal, K.K., Baseer,

V.V. (Eds.), Captive breeding for aquaculture and fish germplasm conservation, NBFGR-NATP Publ. 3: 1-11.

Hartling, R.C., Kunkel, J.G. 1999. Developmental fate of the yolk protein lipovitellin in embryos and larvae of winter flounder, *Pleuronectes americanus*. *Journal of Experimental Zoology*, 284: 686-695.

Hibiya, T. 1982. An atlas of fish histology: normal and pathological features. 2nd Edn., Kodansha Ltd., Tokyo. PP: 104-110.

Hilbert, D.W., Bradford. M., Parker, T., Westcott, D.A. 2004. Golden bowerbird (*Prionodura newtonia*) habitat in past, present and future climates: predicted extinction of vertebrate in tropical highlands due to global warming. *Biological Conservation*, 116: 367–377.

Hislop, J.R.G. 1988. The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British water. *Journal of Fish Biology*, 32: 923-930.

Hogg, R.G. 1976. Established exotic cichlid fishes in dade county, Florida. *Florida Scientist*, 39 (2): 97-103.

Hojo, R.E.S., Santos, G.B., Bazzoli N. 2004. Reproductive biology of *Moenkhausia intermeida* (Eigenmann) (Pisces, Characiformes) in Itumbiara Reservoir, Goias. *Brazilian Revista Brasileira de Zoologia*, 21: 519–524.

Hoque, M.A., Hossain, M.A. 1993. Sexual maturity and fecundity of the fresh water catfish *Mystus vittatus* (Bloch). *Rajshahi University Journal of Zoology*, 12: 9-13.

Hora, S.L. 1929. An aid to the study of Hamilton-Buchanan's ''Gangetic fishes''. *Mem Indian Museum*, 9: 169–192.

Hora, S.L. 1940. On a collection of fish from the headwaters of the Mahanadi river, Raipur district, U.P. *Records of Indian Museum*, 42(2): 365-374.

Hossain, M.A., Taleb, A., Rahman, M.H. 1992. Reproduction and fecundity of *Ompok pabda* (Ham.). *Bangladesh Journal of Scientific Research*, 101: 49-52.

Hossain, Md., Yeamin, Rahman, Md.M., Elgorban, A. 2012. Relationships between body size, weight, condition and fecundity of the threatened Fish, *Puntius ticto* (Hamilton, 1822) in the Ganges River, Northwestern Bangladesh. *Sains Malaysiana*, 41(7): 803–814.

Hossain, Q., Hossain, M.A., Parween, S. 2006. Artificial breeding and nursery practices of *Clarias batrachus* (Linnaeus, 1758). *Scientia World,* 4: 32-37.

Htun-Han, M. 1978a. The reproductive biology of the dab *Limanda limanda* (L.) in the North Sea: Seasonal changes in the ovary. *Journal of Fish Biology,* 11: 293-308.

Htun-Han, M. 1978b. The reproductive biology of dab, *Limanda limanda* in the North Sea: seasonal changes in the ovary. *Journal of Fish Biology,* 13: 351-359.

Hunter, J.R., Macewicz, B.J. 1985. Rates of atresia in the ovary of captive and wild Northern anchovy *Engraulis mordex*. *Fisheries Bulletin*, 83(2): 119-136.

Hunter, J.R., Macewicz, N.C. Lo., Kimbrell, C.A. 1992. Fecundity, spawning and maturing of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fisheries Bulletin of United States,* 90: 101-128.

Hunter, J.R., Macewicz. B.J., Sibert, J.R. 1986. The spawning frequency of skip jack tuna, *Katsuwonus pelamis*, from the south pacific. *Fisheries Bulletin,* 84: 895-903.

Hussain, A. 2006. Seed production of *Ompok pabda* (Ham) in hatchery of C.G. Co-operative Fish Federation. *Fishing Chimes*, 26(1): 136 – 139.

Hussain, M.A., Khatun, M.A., Siddique, M.A.B., Flowra, F.A., Alam, M.M., Sultana, S. 2012. Morphometric characters of freshwater fish *Xenentodon cancila* collected from Rajshahi city, *Bangladesh Journal of Biological Science,* 20: 171-177.

Huxley, L.S. 1924. Constant differential growth-ratios and their significance. *Nature*, 114: 895-896.

Inyang, N. M., Hettiarachchi, M. 1994. Efficiency of human chorionic gonadotropin (HCG) and crude extract of fish and frog in oocyte maturation and ovulation in African catfish *Clarius gariepinus* (Burchell). *Aquaculture Fish Management,* 25: 248-258.

Isa, M.M., Noor, N.S., Yahya, K., Nor, S.A. 2012. Reproductive biology of estuarine catfish, *Arius argyropleuron* (Siluriformes: Ariidae) in the northern part of Peninsular Malaysia. *Journal of Biology, Agriculture and Healthcare,* 2(3): 14-27.

IUCN. 2010. Red list of threatened species. http://dx.doi.org/10.2305/IUCN.UK.2010-4.RLTS.T166616A6248140.en.

Iversen, E.S. 1996. Living marine resources: Their utilization and management. Chapman and Hall, New York, USA, ISBN-13: 9780412987410, Pages: 403.

Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (ed.). 1997. Fish stress and health in aquaculture. Soc. Exp. Biol. Sem. Ser. 62. Cambridge Univ. Press, Cambridge, U.K.

Jadhav, P.G., Bapat, S.S. 1983. Studies on maturity and spawning of *Nemacheilus (noemacheilus) botia* (Ham.Buch.). *Journal of Biological Science,* 22: 125-132.

Jain, S.K., Agarwal, P.K., Singh, V.P. 2007. Krishna and Godavari Basins. Hydrol. Water Resour. India, 57: 641-699.

Janssen, P.A.H., Lambert, J.D.G., Goos, H.J.Th. 1995. The annual ovarian cycle and the influence of pollution on vitellogenesis in the flounder, *Pleuronectes flesus*. *Journal of Fish Biology.,* 47: 509-523.

Jayaram, K.C. 1999. The fresh water fishes of the Indian region. Narendra publishing house, Delhi. 27: 551, 18pls.

Jayaram, K.C. 1982. The Cauvery river ecosystem and the patterns of its fish distribution. *Bulletin of Zoological Survey of India,* 4(3): 289- 299.

Jennings, S., Kaiser, M.J., Reynolds, J.D. 2001. Marine Fisheries Ecology. Blackwell Science Publication, Oxford, pp 417.

Jobling, S., Coey, S., Whitmore, J.G., Kime, D.E., Vanlook, K.J.W. 2002. Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Bioogy of Reproduction,* 67: 515-524.

Joshi, K.D., Joshi, P.C. 1989. Seasonal cycle of testicular maturation and interstitial cell activity in the hill-stream teleost *Puntius dukai* (Day). *Indian Journal of Fisheries,* 36(1): 58-64.

Kahkesh F.B., Feshalami, M.Y., Amiri, F., Nickpey, M. 2010. Effect of ovaprim, ovatide, HCG, LHRH-A2, LHRH-A2+CPE and carp pituitary in benni (*Barbus sharpeyi*) artificial breeding. *Global Veterinaria,* 5: 209–214.

Kamisaka, Y., Tagava, M., Tanaka, M. 1999. Semi-annual Reproductive cycle of a small Flounder *Tarphops oligolepis* in Wakasa Bay. *Fisheries Science,* 65: 98-103.

Karmakar, A.K., Das, A., Banerjee, P.K. 2008. Fish Fauna of Subamarekha River. *Records of zoological survey of India,* Occ. Paper No., 283: 1-57.

Kasheif-El, M.A., Shalloof, K.A.Sh., Authman, M.M.N. 2013. Studies on some reproductive characters of Tilapia species in Damietta branch of river Nile, Egypt. *Journal of Fisheries and Aquatic Science*, 1-17.

Khallaf, E.A., Authman, M. 1991. Growth and mortality of *Bargus bayad* (Forskal) in Bahr Shebeen Canal. *Journal of the Egyptian-German Societyof Zoology*, 4: 87-109.

Khan, M.S.A., Alam, M.J., Rehman, S., Mondal, S., Rahman, M.M. 2002. Study on the gonadosomatic index and fecundity of brackish water catfish, *Plotosus canius* (Ham.-Bucch). *Online Journal of Biological Science*, 2: 232-234.

Khan, H. 1938. Ovulation in fish (Effect of administration of anterior lobe of pituitary gland). *Current Science*, 7(5): 233-234.

Khan, M.M.R., Mollah M.F.A. 1998. Embryonic and larval development of African catfish, *Clarias gariepinus* (Burchell). *Bangladesh Journal of Fisheries*, 21: 91-97.

Khanna, S.S., Pant, M.C. 1996. Structure and seasonal changes in the testes of a hill stream fish *Glyptosterunm pectinopterus*. *Japan Journal of Ichihyology*, 14: 110-119.

King, M. 1995. Fisheries Biology: Assessment and Management. Fishing news Books publication, Oxford, UK p. 341

Kiran, B.R., Shankar, M.K., Venkateshwarlu, M. 2013. A review on induced breeding of cat fishes, murrels and climbing perches in India. *Advances in Applied Science Research*, 4(4): 310-323.

Knight, J.T., Butler, G.L., Smith, P.S., Wager, R.N.E. 2007. Reproductive biology of the endangered oxyleyan pygmy perch *Nannoperca oxleyana*, Whitley. *Jornal of Fish Biology*, 71: 1494-1511.

Koc, N.D., Aytekin, Y., Yuce. R. 2008. Ovary maturation stages and histological investigation of ovary of the zebra fish (*Danio rerio*). *Brazilian Archives of Biology and Technology*, 51: 513–522.

Kohinoor, A.H.M., Jahan, D.A., Khan, M.M., Islam, M.S., Hussain, M.G. 2012. Reproductive biology of black feather chital, *Notopterus chitala* cultured in a

pond of Bangladesh. *International journal of Agricultural Research, Innovation and Technology*, 2(1): 26-31.

Kohler N., Casey, J., Turner, P. 1995. Length- weight relationships for 13 species of sharks from the western North Atlantic. *Fishery Bulletin*, 93: 412-418.

Kovac, V., Copp, G.H. 1999. Morphometry of the stone loach *Barbatula barbatula* (L.). Do metric characters re- flect the species' life history threshold? *Environmental Biology of Fish*, 56: 105 – 115.

Koya, Y., Hamatsu, T., Matsubara, T. 1995. Annual reproductive cycle and spawning characteristics of female Kichiji rock fish *Sebastolobus macrochir*. *Fisheries Science*, 61: 203-208.

Krishnamurti, C.R., Bilgrami, K.S., Das. T.M., Mathur. R.P. 1991. The Ganga, a scientific study. Ganga Project Directorate. Northern Book Center, New Delhi.

Kulshrestha, S.K., Adholia, U.N., Bhatnagar, A., Khan, A.A. 1990. Length weight relationship *Wallago attu* (Schneider) with reference to certain environmental conditions from selected river of Madhya Pradesh and Rajasthan. *Environment and Ecology*, 8(4): 1190-1194.

Kumar, A., Singh, I.J., Ram, R.N. 2003. Annual reproductive cycle of male rohu, *Labio rohita* (Ham) In Tarai region Uttaranchal. *Indian Journal of Fisheries*, 50: 231-241.

Kumar, K., Mohanty, U.K., Dasgupta, S., Sahu, A.K. 2010. Induced spawning of Anabus (*A. testudineus*, Bloch) under captivity in pre-monsoon and monsoon months. *Journal of the Inland Fisheries Society of India*, 42(2): 8-13

Kumari, S. 2014. Study on Growth and Reproductive Behavior in Freshwater Catfish (*Heteropneustes fossilis*). *Sch. Acad. J. Biosci.,* 2(6): 380-383.

Kume, G., Furumitsu, K.,, Yamaguchi, A. 2008. Age, growth and age at sexual maturity of fan ray *Platyrhina sinensis* (Batoidea: Platyrhinidae) in Ariake Bay, *Japan. Fisheries Science*, 74(4): 736–742.

Lagler, K.F. 1956. Freshwater fishery biology. Second Ed. W.M.C. Brown Company, Dubuque, lowa, 421 pp.

Lakra, W.S, Sarkar, U.K., Gopalakrishnan, A., Pandian, A.K. 2010a. Threatened freshwater fishes of India. NBFGR publication, National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh, India, ISBN: 978-81-905540-5- 3, 268.

Lakra, W.S., Sarkar, U.K., Kumar, R.S., Pandey, A., Dubey, V.K., Gusain, O.P. 2010b. Fish diversity, habitat ecology and their conservation and management issues of a tropical River in Ganga basin, India. *Environmentalist.*, 30: 306–319.

Lam, T.J. 1983. Environmental influences on gonadal activity in fish. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (eds) Fish physiology. Reproduction. Part B. Behavior and fertility control, vol IX. Academic Press, New York, pp 65–101

Lamba, V.J., Shashi, V.G., Sundararaj, B.I. 1983. Circannual and circadian variations in plasma levels of steroids (Cortisol, Estradiol-17β, Estrone, and Testosterone) correlated with the annual gonadal cycle in the catfish, *Heteropneustes fossilis* (Bloch). *General and Comparative Endocrinology*, 50: 205–225.

Lawson, E.O. 2011.Testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* (Bloch & Schneider) from Lagos lagoon, Nigeria. *Americal Journal of Science*, 7: 48-59.

Le Cren, E.D. 1951. The length–weight relationship and seasonal cycle in gonad weight and condition in the perch *Perca fluviatilis*. *Journal of Animal Ecology,* 20: 201–219.

Legendre, M. 1986. Seasonal changes in sexual maturity and fecundity and HCG Induced breeding of the catfish *Heterobranchus longifilis* Vol. (Flarridae) reared in Ebrie Lagoon (Ivory coast). *Aquaculture,* 55: 201-213.

Lehri, G.K. 1968. The annual cycle in the ovary of the cat fish, *Clarias batrachus*, L. *Acta Anatomica (Basel),* 69: 105-124.

Leonardos, I.D., Tsikliras, A.C., Batzakas, I., Ntakis, A., Liousia, V. 2009. Life-history characteristics of the endangered Aristotles catfish (*Silurus aristotelis* Garman, 1890), Lake Pamvotis, north-western Greece. *Journal of Applied Ichthyology*, 25: 746–751.

Lima-Junior, S.E., Cardone, I.B., Goitein, R. 2006. Fish assemblage structure and aquatic pollution in a Brazilian stream: some limitations of diversity indices and models for environmental impact studies. *Ecology of Freshwater Fish*, 15: 284-290.

Loh, J.Y., Ting, A.Y. 2015. Comparative study of analogue hormones and embryonic, larvae and juvenile development on the induced breeding of climbing perch, (*Anabus testitudineus*, Bloch, 1792). *International Journal of Fisheries and Aquatic Studies*, 2: 277-278.

Love, R.M. 1970. The chemical Biology of fishes. Academic press, Landon.

Lowe-McConnell, R.H. 1982. Tilapia in fish communities. In Pullin RVS, Lowe McConnell RH (Eds) Proceedings of the International Conference on the Biology and Culture of Tilapias (1): 83-113.

Lowry, O.H., Rosebrough, N., Farr, A.L., Randall, R.J. 1951. Protein measurement with Folin Phenol reagent. *Journal of Biological Chemistry*, 193: 265-285.

Lubzens, E., Young, G., Bobe, J., Cerda, J. 2010. Oogenesis in teleosts: How fish eggs are formed. *General and Comparative Endocrinology*, 165: 367-389

Luzzana, U., Serrini, G., Moretti, V.M., Grimaldi, P., Paleari, M.A., Valfre, F. 1996. Seasonal variations in fat content and fatty acid composition of male and female Coreganid bondella from Lake Maggiore and Land locked shad from Lake Cemo (North Italy). *Journal of Fish Biology*, 48 (3): 352-366.

Macay, M., Tunison, A.V. 1936. Cortland Hatchery Report No. 5. N.Y. State Cons. Deptt. Us Bur of fish and Cornell Univ.

Machado-Allison, A. 1987. Los peces de los Llanos deVenezuela. Universidad Central de Venezuela, Caracas, Universidad Central de Venezuela, pp 144.

Machado-Allison, A. 1992. Larval ecology of fish of the Orinoco Basin. In: Hamlett WC (ed) Reproductive biology of South American Vertebrates. Springer Verlag, New York, pp 45

Machado-Allison, A. 1994. Factors affecting fish communities in the flooded plains of Venezuela. *Acta Biologica Venezuelica*, 15: 59–75

MacKenzie, D., Thomas, P., Farrar, S.M. 1989. Seasonal changes in thyroid and reproductive steroid hormones in female channel catfish (*Ictalurus punctatus*) in pond culture. *Aquaculture*, 78: 63–80.

Maclean, R.H., Jones, R.W. 1995. Aquatic Biodiversity Conservation: a review of current issues and efforts. Ottawa. ON. SIFR, pp 56.

Magalhaes, A.L.B., Andrare, R.F., Gomes, B.V.C., Perini, V.R., Rizzo, E., Bazzoli, N. 2011. Ultrastructure of the semicystic spermatogenesis in the South American freshwater characid *Hemigrammus marginatus* (Teleostei, Characiformes). *Journal of Applied Ichthyology,* 27: 1041-1046.

Mahapatra, B.K. 2004. Conservation of the Asiatic catfish, *Clarias batrachus* through artificial propagation and larval rearing technique in Imdia. *Aquaculture,* 9 (4): 8-9.

Mahmood, S.U. 2006. Effects of different feeds on the larval rearing of Pabda, *Ompok pabda. Bangladesh Journal of Zoology,* 34: No.1.

Malhotra, Y.R. 1963. On the nucleolar extrusion in the developing oocytes of a Kashmir fish, *Schicothorax niger*. Ichthyologica, 2: 57-60.

Malla, S., Banik, S. 2010. Rotifer as promoter for enhancing growth rate and survival potential for a rare fish *Ompok bimaculatus* In: Banik, S, (ed) *Proceeding of Aquatic Biotechnology*, 18-28.

Malla, S., Banik, S. 2015. Reproductive biology of an endangered catfish, *Ompok bimaculatus* (Bloch, 1794) in the lotic waterbodies of Tripura, North-East India. *International Journal of Fisheries and Aquatic Studies,* 2(4): 251-260.

Manickam, P., Joy, K.P. 1989. Induction of maturation and ovulation by pimozide-LHRH analogue treatment and resulting high quality egg production in the Asian catfish, *Clarias batrachus* L. *Aquaculture,* 83: 193–199.

Manna, A.K., Raut, S.K. 1991. Clutch size in two shrimp species *M. lamarres* and *M. day*. Anum. *Environment and Ecology,* 9: 349-351.

Manna, M.M., Maridass, M., Thangarani, S. 2010. Gonad developmental cycle of *Puntius filamentous. International Journal of Bio-Science and Bio-Technology,* 1(2): 69-77.

Marcano, D., Cardillo, E., Rodrıguez, C., Poleo, G., Gago, N., Guerrero, H.Y. 2007. Seasonal reproductive biology of two species of freshwater catfish from the Venezuelan flood-plains. *General and Comparative Endocrinology,* 153: 371–377.

Marimuthu, K., Haniffa, M.A. 2007. Embryonic and Larval Development of the Striped Snakehead *Channa striatus*. Taiwania, 52(1): 84-92.

Marza, V.D. 1938. Histophysiologie de l'Ovogenese. Herman, Paris.

Mas-Marti, E., Garcia-Berthou, E., Sabater, S., Tomanova, S., Monoz, I. 2010. Comparing fish assemblages and trophic ecology of permanent and intermittent researches in a Mediterranean stream. *Hydrobiologia* 657(1): 167-180.

Mattei, X., Siau, Y., Thiaw, O.T., Thiam, D. 1993. Peculiarities in the organisation of testes of *Ophidion* spp.(Pisces Teleostei), Evidence for two types of spermatogenesis in teleost fish. *Journal of Fish Biology*, 43: 931-937

Matthews, W.J. 1998. Patterns in freshwater fish ecology. New York, Chapman & Hall, 756p.

Maurice, Burton. 1984. Encyclopedia of Fish. Americal Museum of Natural History, Washington, USA.

Mayer, I., Shackley, S.E., Ryland, J.S. 1988. Aspects of the reproductive biology of the bass Dicentrarchus labraxl An Histochemical study of oocyte development. *Journal of Fish Biology*, 33: 609-622.

Mazzoni, R., Caramaschi, E.P., Fenerich-Verani, N. 2002. Reproductive biology of a characidiinae (Osteichthyes, Characidae) from the Ubatiba River, Marica´–RJ. *Brazilian Journal of Biology*, 62: 487–494.

Mazzoni, R., Silva, A.P.F. 2006. Aspectos da história de vida de *Bryconamericus microcephalus* (Miranda Ribeiro) (Characiformes, Characidae) de um riacho costeiro de Mata Atlântica, Ilha Grande, Rio de Janeiro. *Brazil Revista Brasileira de Zoologia*, 23(1): 228-233.

McLaughlin R.L., Grant J.W.A. 1994. Morphological and behavioral differences among recently-emerged brook charr, *Salvelinus fontinalis*, foraging in slow- vs. fast-running water. *Environemtal Biology of Fishes*, 39: 289–300.

Mekkawy, I.A.A., Hassan, A.A. 2011. Some reproductive parameters of *Synodontis schall* (Bloch and Scchneider, 1801) from the river Nile, Egypt. *Journal of Fisheries and Aquatic Science*, 6: 456-471.

Menezes, N.A., Vazzoler, A.E.A.M. 1992. Síntese de conhecimento sobre o comportamento reprodutivo dos Characiformes da América do Sul (Teleostei, Ostariophysi). *Revista Brasileira de Biologia*, 52(4): 627-640.

Mir, J.I., Sarkar, U.K., Dwivedi, A.K., Gusain, O.P., Jena, J.K. 2013. Stock structure analysis of *Labeo rohita* (Hamilton, 1822) across the Ganga basin (India) using a truss network system. *Journal of Applied Ichthyology*, 29: 1097-1103.

Miranda, L.A., Strussmann, C.A., Somoza, G.M. 2009. Effects of light and temperature conditions on the expression of GnRH and GtH genes and levels of plasma steroids in *Odontesthes bonariensis* females. *Fish Physiology and Biochemistry*, 35: 101-108.

Mishra, A., Rawat, A., Verma, S., Sarkar, U.K., Kumar, R. 2016. Spawning dynamics of freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in different major rivers of India. *International Journal of Fisheries and Aquatic Studies*, 4(3): 343-345

Mishra, S., Saksena, D.N. 2012. Gonadosomatic index and fecundity of an Indian major carp, *Labeo calbasu* in gonad reservoir. *The Bioscan*, 7(1): 43-46.

Mishra, S.K., Sarkar, U.K., Trivedi, S.P., Mir, J.I., Pal A. 2013. Biological parameters of a silurid catfish *Ompok bimaculatus* (Bloch, 1794) from River Ghaghara, India. *Journal of Environmental Biology*, 34: 1013-1017.

Mohamed, A.A. 2010. The reproductive biology and the histological and ultrastructural characteristics in ovaries of the female gadidae fish *Merluccius merluccius* from the Egyptian Mediterranean water. *African Journal of Biotechnology*, 9: 2544–2559.

Mohammadian, T., Malekpouri, P., Zare, M., Zainodini, M.A. 2015. Effects of different spawning agents on serum levels of reproductive steroid hormones and cortisol level in adult female *Barbus sharpeyi* (Gunther, 1874). *Fish Physiology and Biochemistry*, 41(6): 1475-89.

Mohan, D., Jhajhria, A. 2001. Comparative growth and production studies of carps in Skekhawat pond Jodhpur. *Journal of Natcon*, 13(2): 123-130.

Molen, S.V.D., Matallanas, J. 2004. Reproductive biology of female Antarctic spiny plunderfish *Harpagifer spinosus* (Notothenioidei: Harpagiferidae) from Iles Crozet. *Antarctic Science*, 16(2): 99–105.

Mollah, M.F.A. 2003. Mass production of stockable sized fry of *Ompok pabda* (Hamilton). University link, No-3.

Mollah, M.F.A., Tan, E.S.P. 1983. HCG–induced spawning of the catfish, *Clarius macrocepalus* (Gunther). *Aquaculture*, 35: 239–247.

Mollet, H.F., Cliff, G., Pratt, H.L., Jr. Stevens, J.D. 2000. Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. US. *Fishery Bulletin*, 98: 299-318.

Morato, T.P., Afonso, P., Lourinho, P., Barreiros, J.P., Santos, R.S., Nash, R.D.M. 2001. Length-weight relationships for 21 coastal fish species of the Azores, north-eastern Atlantic. *Fisheries Research*, 50: 297-302.

More, P.R., Bhandare, R.Y., Shinde, S.E., Pathan, T.S., Sonawane, D.L. 2010. Comparative Study of synthetic hormones ovaprim and carp pituitary extract used in induced breeding of Indian major carps. *Libyan Agriculture Research Center Journal International*, 1 (5): 288-295.

Morris, J.A., Sullivan, C.V., Govoni, J.J. 2011. Oogenesis and spawn information in the invasive lionfish, *Pterois miles* and *Pterois volitans*. *Scientia Marina*, 75: 147–154.

Moyle, P.B., Cech, J.J. 2000. Fishes: An introduction to ichthyology. 4th Edn., Prentice Hall, Upper Saddle River, New Jersey, USA., ISBN-13: 9780130112828, Pages: 612.

Mukherjee, M., Das, S. 2001. Artificial propagation of a siluroid fish *Ompok pabda* (Ham). *Fishing Chimes*, 26 (1): 75–79.

Mukhopadhyay, S., Sinha, G.M. 1986. Annual cyclical changes in the testicular activity of an Indian fresh water major carp *Labeo rohita* (Ham.). *Gegenbaurs Morphologisches Jahrbuch*, 132: 303-321

Murthy, K.S., Ventakateshwaralu, M., Kiran, B.R. 2013. Induced breeding of Indian major, minor and cyprinid carps in India: An overview. *Journal of International Academic Research for Multidiscipilinary*, 1(6): 165-193.

Murua, H., Kraus, G., Saborido-Rey, F., Witthames, P.R., Thorsen, A., Junquera, S. 2003. Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. *Journal Northwest Atlantic Fishery Science*, 33: 33-54.

Mustafa, G., Islam, K.R., Ali, S., Alam, A.K.M.A.A. 1983. Some aspects of the biology of *Puntius sarana* (Ham.): Food and feeding habits in relation to fish size and fecundity. *Bangladesh Journal of Zoology*, l0 (2): 92-100.

Naeem, M., Salam, A., Ashraf, M., Khalid, M., Ishtiaq, A. 2011a. External morphometric study of hatchery reared Mahseer *Tor putitora* in relation to Body size and condition factor from Pakistan. *African Journal of Biotechnology*, 10(36): 7071-7070.

Naeem, M., Salam, A., Gillani, Q., Ishtiaq, A. 2010a. Length-weight relationships of Notopterus notopterus and introduced *Oreochromis niloticus* from the Indus River, southern Punjab, Pakistan. *Journal of Applied Ichthyology*, 26: 620.

Naeem, M., Salam, A., Ishtiaq, A. 2011b. Length-weight relationships of wild and farmed Tor putitora from Pakistan. *Journal of Applied Ichthyology*, 27(4): 1133-1134.

Naeem, M., Salam, A., Ishtiaq, A., Shafique, A. 2010b. Length-weight and condition factor relationship of farmed hybrid (*Catla catla*♂ X *Labeo rohita*♀) from Multan, Pakistan. *Sindh University Research Journal. (Sci. Ser.)*, 42(2): 35-38.

Nagahama, Y. 1983. The functional morphology of teleost gonads. In: Fish Physiology Reproduction, edited by Hoar WS Randall DJ and Donaldson EM (New York Academic Press), 9A: 223-275.

Nandeesha, M.C., Bhadraswamy, G., Patiil, J.G., Varghese, T.J., Sharma, K., Keshavanath, P. 1993. Preliminary results on induced spawning of pond-raised mahaseer *Tor khudree*. *Journal of Auqaculture in the Tropics*, 8: 55-60.

Nandeesha, M.C., Das, S.K., Nathaniel, D.E., Varghese, T.J. 1990a. Project report on breeding of carps with ovaprim in India. Special Publication No. 4, *Asian Fisheries Society*, Indian Branch, Mangalore, India.

Nandeesha, M.C., Das, S.K., Nathaniel, D.E., Varghese, T.J., Shetty, H.P.C. 1990b. Breeding of carp with ovaprim standardization of dosage and nation wide demonstration (abstract). World Aquaculture Society, Halifax, Canada. 81.

Nandeesha, M.C., Rao, K.G., Jayanna, R., Parker, N.C., Varghese, T.J., Keshavanath, P., Shetty, H.P.C. 1990c. Induced spawning of Indian major carps through single

application of Ovaprim C. pp. 581- 585. In: Hirano, R., Hanyu, L. (Eds.). The second Asian Fisheries Forum, Asian Fisheries Society, Manila, Philiphines.

Nandikeshwari, R., Anandan, V. 2013. Analysis of gonadosomatic index and fecundity of *Terapon puta* from Nallavadu coast Pondicherry. *International Journal of Scientific Research and Publication,* 3: 1-4.

Neat, F.C., Lengkeek, W., Westerbeek, E.P., Laarhoven, B., Videler, J.J. 2003. Behavioural and morphological differences between lake and river populations of Salaria fluviatilis. *Journal of Fish Biology*, 63: 374–387.

Nejedli, S., Petrinec, Z., Kuzir, S., Srebocan, E. 2004. Annual oscillation of ovarian morphology in European pilchard (Sardina pilchardus, Walbaum) in the Northern Andriatic Sea. *Veterinarski Arhiv*, 74: 97–106.

Nelson, J.S. 2006. Fishes of the World, 4th Edition. John Wiley and Sons, Inc, pp: 601.

Ng, H.H., Tenzin, K., Pal, M.. 2010. Ompok bimaculatus. The IUCN Red List of Threatened Species. 2010:e.T166616A6248140.

Nikolskii, G.V. 1963. The ecology of fishes. London and New York: Academic Press. 325 pp.

Nikolskii, G.V. 1969. Theory of fish population dynamics as the biological background for rational exploitation and management of fishery resources. Edinburg, Oliver & Boyd Ltda, 323p.

Nobrega, R.H., Quagio-Grassiotto, I. 2007. Morphofunctional changes in leydig cells throughout the continuous spermatogenesis of the freshwater teleost fish, *Serrasalmus spilopleura* (Characiformes, Characidae): An ultrastructural and enzyme study. *Cell and Tissue Research*, 329: 339-349.

Ntiba, M.J., Jaccarini, V. 1990. Gonad maturation and spawning times of *Siganus sutor* off the Kenya coast: evidence for definite spawning seasons in a tropical fish. *Journal of Fish Biology,* 37: 315–325.

Nwokoye, C.O., Nwuba, L.A., Eyo, J.E. 2007. Induced propagation of african clariid catfish, *Heterobranchus bidorsalis* (Geoffrey Saint Hillarie, 1809) using synthetic and homoplastic hormones. *African Journal of Biotechnology*, 6(23): 2687-2693.

Parameswaran, S., Selvaraj, C., Radhakrishan, S. 1967. A review of the freshwater fishes of the genus *Ompok. Journal of Zoological Society of India*, 19(1-2): 89-98.

Parameswaran, S., Selvaraj, C., Radhakrishnan, S. 1970. Observations on the biology, induced breeding and cultural possibilities of *Ompak bimaculatus* (Bloch) in ponds. *Proceedings of National Academy of Sciecnces, India* (B), 40: 145-157.

Parua, P.K. 2001. Flood management in Ganga-Brahmaputra Meghna basin: Some aspects of regional co- operation. Retrieved on February 14, 2011 from http://www.uunescap.org/enrd/water_mineral/disaster/flood%20management%20in%20ganga.doc.

Parween, S. 2007. Ompok bimaculatus (Bloch, 1797). In: Siddiqui, K.U., Islam, M.A., Kabir, S.M.H., Ahmed, A.T.A., Rahman, A.K.A., Haque, E.U., Ahmed, Z.U., Begum, Z.N.T., Hasan, M.A., Khondker, M., Rahman, M.M. (eds). Encyclopedia of Flora and Fauna of Bangladesh, vol. 23. Freshwater fishes. *Asiatic Society of Bangladesh*, Dhaka. P. 127.

Pauly, D. 1983. Some Simple methods for the assessment of tropical fish Stock. FAO Fish. Technical Paper.

Pauly, D. 1984. Fish population dynamics in tropical waters: A manual for use with programmable calculators, ICLARM Studies and Reviews 8, *International Center for Living Aquatic Resources Management*, Manila, Philippines, 325 pp.

Pauly, D., Gayanilo, F.C. 1996. Estimating the parameter of length-weight relationships from length frequency samples and bulk weights: 136. In Pauly, D., Martosubroto, P. (eds.) Baseline studies of biodiversity: the fish resources of western Indonesia. *International Center for Living Aquatic Resources Managemnt,* Stud. Rev., 23: 321.

Payne, A.I., Sinha, R.K,. Singh, H.R., Haq, S. 2004. A review of the Ganges Basin: its fish and fisheries. In: Welcomme, R.L., Peter T (eds) Proceedings of the second international symposium on the management of large rivers for Fisheries, vol 1, FAO Regional Office for Asia and the Pacific, Bangkok, Thailand, pp 229–251

Pervin, M.R., Mortuza, M.G. 2008. Notes on length-weight relationship and condition factor of freshwater fish, *Labeo boga* (Hamilton) (Cypriniformes: Cyprinidae). *University Journal of Zoology, Rajshahi University*, 27: 97-98.

Peter, R.E., Sokolowska, M., Nahorniak, C.S. 1986. Comparison of (D-Arg^{6}Trap^{7},Leu^{8}, Pro9Net) luteinising hormone (LHRA) in combination with pimozide, in

stimulating gonadotropin release and ovulation in the gold fish, *Carrassius auratus*. *Canadian Journal of Zoology*, 65: 987-91.

Peter, R.E., Yu, K.L. 1997. Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Reviews in Fish Biology and Fisheries*, 7:173–197.

Petrakis, G., Stergiou, K.I. 1995. Weight-length relationships for 33 fish species in Greekwaters. *Fisheries Research,* 21: 465-469.

Purkayastha, S., Sarma, S., Gupta, S., Singh, A.S., Biswas, S.P. 2012. Captive breeding of an endangered fish *Ompok Pabda* (Hamilton -Buchanan) with Ovatide from Guwahati, Assam. *Asian Journal of Experimental Biological Sciences*, 3(2): 267-271.

Quagio-Grassiotto, I., Guimaraes, A.C.D. 2003. Follicular epithelium, theca and egg envelope formation in *Serrasalmus spilopleura* (Teleostei, Characiformes, Characidae). *Acta Zoologica,* 84: 121-129.

Raghuveer, K., Senthilkumaran, B. 2010. Cloning and differential expression pattern of vasa in the developing and recrudescing gonads of catfish, *Clarias gariepinus*. *Comparative Biochemistry and Physiology- Molecular and Integrative Physiology,* 157 (1): 79-85.

Rahman, A., Yeasmine, S., Khan, M.M. 2008. Growth, survival and production of a threatened siluried catfish, *Ompok pabda* (Hamilton) fingerlings in nursery ponds. *Malaysian Applied Biology,* 37 (1): 47-56.

Rahman, A.K.A. 1989. Freshwater fishes of Bangladesh. The Zoological Society of Bangladesh, Department of Zoology, University of Dhaka, Dhaka-1000., pp 167.

Rahman, A.K.A. 2005. Freshwater fishes of Bangladeah (Second edition). The Zoological Socity of Bangladesh, Department of Zoology, University of Dhaka, Dhaka-1000, pp 394.

Rahman, M.M., Hossain, M.Y., Hossain, M.I., Rovhat, S.J., Islam, M.S., Hossain, M.B. 2013. Induced breeding of the stinging satfish, *Heteropneustes fossilis*: comparison among different inducing agents introduction. *Turkish Journal of Fisheries and Aquatic Sciences*, 13: 523-527.

Rahman, M.R., Rahman, M.A., Khan, M.N., Hussain, M.G. 2004. Observation on the embryonic and larval development of silurid catfish, gulsa (*Mystus cavasius*). *Pakistan Journal of Biological Sciences,* 7(6): 1070-1075.

Raizada, S., Lal, K.K., Sarkar, U. K., Varshney, P.K., Sahu, V., Yadav, K.C., Agnihotri, P., Awasthi, A., Jena, J. K. 2013. Captive breeding and embryonic development of butter catfish (*Ompok bimaculatus,* Bloch 1794), a threatened fish of Indian sub-continent in Northern India. *Proceedings of National Academy of Sciecnces, India* (B): Biological Science, 83 (3): 333–339.

Raj, B.S. 1962. The extraordinary breeding habits of the catfish Mystus aor (Ham) and *Mystus seenghala* (Sykes). *Proceedings of National Institute of Sciecnces of India*, 28: 193-200.

Rajagopal, B., Davidar, P. 2008. On the population and breeding aspects of catfish in fresh water wetlands of Tamil nadu, peninsular India. *Electronic Journal of Ichthyology*, 1: 18-30.

Ramaswamy, L.S., Sunderraj, B.I. 1969. Induced spawning in the Indian catfish, *Heteropneustes fossilis* with pituitary injection. *Acta Anatomica*, 31: 551-562.

Rao, B.J., Karamchandani, S.J. 1986. On the spawning biology of *Ompok bimaculatus* (Bloch) from Kulgarhi reservoir of Madhya Pradesh. *Journal of the Inland Fisheries Society of India*, 18(2): 40-47.

Rao, C., Krishnan, L. 2009. Studies on the reproductive biology of the female spiny cheek grouper, *Epinephelus diacanthus* (Valenciennes, 1828). *Indian Journal of Fisheries*, 56(2): 87-94.

Rao, K.L. 1979. India's Water Wealth, its Assessment Uses and Projections. Delhi: Orient Longman.

Rao, L.M., Reddy, K.S., Hymavathy, V. 1999. Breeding biology and fecundity in *Mystus* species from Mehadrigedda stream of Vishakapatnam. *Ecology, Environment and Conservation*, 5(1): 25-28.

Renjini, P.K., Nandan, B.S. 2011. Length-weight relationship, condition factor and morphometry of gold spot mullet *Liza parsia* (Hamilton, 1822) from Cochin estuary. *Indian Journal of Geo-Marine Sciences*, 40(4): 567-571.

Revenga, C., Mock, G. 2000.Pilot analysis of global ecosystems: freshwater systems and world resources 1998–99. World Resources Institute, Washington, DC.

Rheman, S., Islam, M.L., Shah, M.M.R., Mondal, S., Alam, M. 2002. Observation on the fecundity and gonadosomatic index (GSI) of the grey mullet *Liza parsia* (Ham.). *Journal of Biosciences*, 2: 690-693.

Ricciardi, A., Rasmussen, J.B. 1999. Extinction rates of North American freshwater fauna. *Conservation Biology*, 13: 1220–1222.

Riehl, R., Baensch, H.A. 1991. Aquarium Atlas. Melle, Germany, Mergus.

Rimmer, M.A., Merrick, J.R. 1983. A review of the reproduction and development in the fork tailed catfishes (Ariidae). J. Limno. Soc. London., 107: 41-50.

Roy, A.K., Sarma, S., Bhuyan, D.K., Das, M., Das, A.K. 2007. Captive breeding of an endangered fish *Ompok pabda* (Hamilton-Buchanan) using different doses of ovatide. In National seminar on recent advances & rebuilding of fish and fisheries in North-East India. Department of Pisciculture, St. Anthony College, Shillong-1 (Abstract).

Roy, P.K., Hossain, M.A. 2006. The fecundity and sex ratio of *Mystus cavasius* (Hamilton) (Cypriniformes: Bagriae). *Journal of Life and Earth Science*, 1(2): 65-66.

Sabadin, D., González-Castro, M., Iudica, C., Díaz de Astarloa, J.M., Fernández Iriart, P.J. 2010. Morphometric and genetic assessment of the *Cynoscion guatucupa* population structure from Buenos Aires coast, Argentine sea. *Revista de Biologia Marina y Oceanografia*, 45: 513-517.

Sacotte S., Magnan P. 2006. Inherited differences in foraging behaviour in the offspring of two forms of lacustrine brook charr. *Evolutionary Ecology Research*, 8: 843–857.

Sahoo, S.K., Giri, S.S., Chandra, S., Mohapatra, B.C. 2008. Evaluation of breeding performance of Asian Catfish *Clarias batrachus* at different dose of HCG and latency period combinations. *Turkish Journal of Fisheries and Aquatic Sciences*, 8: 249-251.

Sahoo, S.K., Giri, S.S., Sahu, A.K. 2005. Effect on breeding performance and egg quality of *Clarias batrachus* (Linn.) at various doses of ovatide during spawning induction. *Asian Fisheries Science*, 18: 77-83

Saifullah, A.S.M., Rahman S. Md., Khan, Y., Ahmed, S. 2004. Fecundity of *Hilsa ilisha* (Ham.) from the Bay of Bengal. Pakistan Journal of Biological Sciences, 7(8): 1394-1398.

Sajina, A.M., Chakraborty, S.K., Jaiswar, A.K., Pazhayamadom, D.G., Sudheesan, D. 2011. Stock structure analysis of Indian Mackerel, *Rastrelliger kanagurta* (Cuvier, 1816) along the Indian coast. *Asian Fisheries Science*, 24: 331-342.

Saksena, D.N. 1987. On the use of gonadosomatic index and volume of gonads as indicators of gonadal state in Indian freshwater goby *Glassogobius giuris* (Ham) with a note on the role of temperature in fish reproduction. *International Journal of Applied Ichthyology*, 8: 1-8.

Salami, A.A., Fagbenro, O.A., Edibite, L., Fagbemiro, S.O. 1994. Induced spawning of the African catfish *Clarias gariepinus* using non–piscine pituitary extracts. *World Aquaculture Society*, 25: 166-168.

Santos, J.E., Bazzoli, N., Rizzo, E., Santos, G.B. 2001. Morpho functional organization of the male reproductive system of the catfish *Iheringichthys labrosus* (Luken, 1874) (Siluriformes: Pimelodidae). *Tissue cell*, 33: 533-540.

Sarkar, P.K., Pal, H.K., Rahman, M.M., Rahman, M.M. 2002. Observation on the fecundity and gonadosomatic index of *Mystus gulio* in brackish waters of Bangladesh. *Journal of Biolgical Sciences*, 2: 235-237.

Sarkar, U. K., Deepak, P. K., Negi, R. S., Singh, S. P., Kapoor, D. 2006. Captive breeding of endangered fishes *Chitala chitala* (Hamilton-Buchanan) for species conservation and sustainable utilization. *Biodiversity and Conservation*, 15(11): 3579- 3589

Sarkar, U.K., Agnihotri, P., Kumar, R., Awasthi, A., Pandey, B.K., Mishra, A. 2017. Dynamics of inter-population reproductive pattern in butter catfish, *Ompok bimaculatus* (Bloch, 1794) from different rivers in India. *Turkish Journal of Fisheries and Aquatic Sciences* (Acepted) ISSN 1303-2712.

Sarkar, U.K., Deepak, P.K., Negi, R.S., Paul, S.K., Singh, S.P. 2005. Captive breeding of and endangered fish *Ompok pabda* (Ham-Buch) using different dosage of Ovaprim. *Journal of the Inland Fisheries Society of India*, 37(2): 37-42.

Sarkar, U.K., Dubey, V.K., Singh, A.K., Gupta, B.K., Pandey, A., Sani, R.K., Lakra, W.S. 2012. The recent occurrence of exotic freshwater fishes in the tributaries of river Ganga basin: abundance, distribution, risk, and conservation issues. *The Environmentalist*, 32(4): 476-484.

Sarkar, U.K., Gupta, B.K., Lakra W.S. 2010. Biodiversity, ecohydrology, threat status and conservation priority of freshwater fishes of River Gomati, a tributary of River Ganga (India). *Environmentalist*, 30: 3–17.

Sarkar, U.K., Negi, R.S., Deepak, P.K., Lakra, W.S., Paul, S.K. 2008. Biological parameters of endangered fish *Chitala chitala* (Osteoglossiformes: Notopteridae) from some Indian rivers. *Fisheries Research*, 90: 170-177.

Sarkar, U.K., Pathak, A.K., Lakra, W.S. 2008a. Conservation of freshwater fish resources of India: new approaches, assessment and challenges. *Biodiversity and Conservation*, 17: 2495–2511.

Sarma, D., Das, J., Dutta, A., 2012a. Management of *Ompok pabo* brooders- a pre requisite strategy for captive breeding. *International Journal of Pharm and Bio Sciences*, 3(2):126-130.

Sarma. D., Das. J., Dutta. A., Goswami, U.C. 2012b. Early embryonic and larval development of *Ompok pabo* with notes on its nursery rearing. *European Journal of Experimental Biology*, 2 (1):253-260.

Sarma. D., Das. J., Goswami, U.C., Dutta, A. 2012. Present Status and Habitat Ecology of *Ompok pabo* (Ham-Buchanan) in Goronga Beel, Morigaon; Assam (India). *Advances in Applied Science Research*, 3 (1): 481-488.

Sathyanesan, A.G. 1959. Seasonal histological changes in the testes of the cat fish (*Mystus seenghala*). *Journal of the Zoological Society of Indai*, 11: 52-59.

Sathyanesan, A.G. 1962. The ovarian cycle in the catfish, *Mystus seenghala*. *Proceedings of National Institute of Sciecnces of India*, 28: 497-506.

Sato, T., Yoseda, K., 2008. Reproductive season and female maturity size of coconut crab *Birgus latro* on Hatoma Island, Southern Japan. *Fisheries Science*, 74 (6): 1277-1282.

Sato, Y., Fenerich-Verani, N., Nuner, A.P.O., Godinho, H.P., Verani, J.R. 2003. Padroes reprodutivos de peixes da bacia do Sao Francisco. In: Aguas, peixe e pesca no Sao Francisco das Minas Gerais. H. P. Godinho and A. L. Godinho (Eds). Pontifi´cia Universidade Cato´lica de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, pp. 229–274.

Selman, K., Wallace, R. 1986. Gametogenesis in *Fundulus heteroclitus*. *American Zoologist*, 26: 173-192.

Sfakianakis, D.G., Leris, I., Laggis, A., Kentouri, M. 2011. The effect of rearing temperature on body shape and meristic characters in zebrafish (*Danio rerio)* juveniles. *Environmental Biology of Fishes*, 92: 197-205.

Shabanipour, N., Hossayni, S.N. 2010. Histological and ultrastructural study of zona radiata in oocyte of common carp *Cyprinus carpio* (Linnaeus 1758). Micron, 41: 877-881.

Shafi, M., Quddus, M.A. 1974. The Fecundity of the common punti, *Puntius stigma* (Cuvier & Valenciennes) (Cyprinidae: Cypriniformes), *Bangladesh Journal of Zoology*, (2): 133- 145.

Shankar, D.S., Kulkarni, R.S. 2005. Somatic condition of the fish, *Notopterus notopterus* (Pallas) during phases of the reproductive cycle. *Journal of Environmental Biology*, 26(1): 49-53.

Sharaf, S.M. 2012. Effect of GnRHa, pimozide and Ovaprim on ovulation and plasma sex steroid hormones in African catfish *Clarias gariepinus*. *Theriogenology*, 77(8): 1709-16.

Sharma, K., Yadava, N.K., Jindal, M. 2010. Effect of different doses of ovatide on the breeding performance of *Clarias batrachus* (Linn.). *Livestock Research for Rural Development*, 22(4): 69.

Shashi, B.S., Akela, B.P. 1996. Determination of maturity and spawning Period by gonadosomatic index and measurement of mean ova diameter In certain teleosts. *Environment and Ecology*, 14: 399-403.

Shein, N.L., Chuda, H., Arakawa, T., Mizuno, K., Soyano, K. 2004. Ovarian development and final oocyte maturation in cultured seven band grouper *Epinephelus septemfasciatus*. *Fisheries Science*, 70: 360-365.

Shengde, A.N., Mane, U.H. 2006. Gonadosomatic index and spawning season of cyprinid fish *Cirrhina reba* (Hamilton). *Journal of Aquatic Biology*, 21(10): 127-123.

Shinde, V.R., Md. Rafiq, Sathish, S.V., Kulkarni, R.S. 2002. Fecundity of the freshwater fish *Channa punctatus* (Bloch) from three aquatic bodies. *Journal of Environment*, 23: 429-432.

Shinkafi, B.A., Ilesanmi, B.D. 2014. Effect of varying doses of ovatide on the breeding performance of african catfish (*Clarias gariepinus* Burchell, 1822) in Sokoto, North-western Nigeria. *Asian Journal of Animal Sciecnes*, 8: 56-64.

Shreni, D., Kalpana. 1980. Seasonal variations in the chemical composition of cat fish. *Heteropneustes fossilis* (Bloch). *Proceedings of Indian Academy of Sciences (Animal Science)*, 89(2): 191-196.

Siddiqua, K.A., Islam, M.S., Hossain, M.G., Ahmed, A.T.A. 2000. A histological study of the spermatogenesis in *Ompok pabda*. *Bangladesh Journal of Fisheries Research*, 4 (2): 185-189.

Siddiqui, A.Q. 1977. Reproductive biology, length weight relationship and relative condition of *Tilapia leucosticta* (Trewaevas) in lake Naivasha Kenya. *Journal of Fish Biology*, 10: 251-260.

Siegfried, C.A. 1980. Seasonal abundance and distribution of Crangon franciscorum and *Palaemon macrodactylus* (Decapoda,Caridea) in the San Francisco Bay-Delta. *The Biology Bulletin*, 159: 177-192.

Singh, K.P., Malik, A., Mohan, D., Sinha. S. 2004. Multivariate Statistical Techniques for the evaluation of spatial and temporal variation in water quality of Gomti River (India). A case study. *Water Research*, 38(18): 3980-3992.

Singh, M. 1996. The Ganga River: fluvial geomorphology, sedimentation processes and geochemical studies. (Heidelberg: Heidelberger Beitr.Umwelt-Geochemi) 133.

Singh, S., Singh, T.P. 1987 Seasonal profiles of sex steroids in blood plasma and ovarian tissue of *Clarias batrachus*. *General and Comparative Endocrionology*, 65:216–224.

Singh, S.P., Mehrotra, S.N., Yadava, Y.S., Choudhury, M., Biswas, B.K., Biswas, D.K. 1988. Investigation on factors relating to decline of fishery in the River Brahmaputra and its tributaries. Annual Report, CICFRI, Barrackpore.

Singh, Y.B., Saha, H., Mandal, B., Tandel, R. 2012. Breeding of climbing perch (*Anabus testudineus* Bloch, 1972) induced with ovatide. *The Isareli Journal of Aquaculture Bamidegeh*, 64: 1-4.

Sinha, R.K. 2006. The Ganges river dolphin *Platanista gangetica*. *Journal of the Bombay Natural History Society*, 103: 254–263

Sivakumaran, K.P., Brown, P., Stoessel, D., Giles, A. 2003. Maturation and reproductive biology of female wild carp, Cyprinus carpio, in Victoria, Australia. *Environmental biology of Fishes*, 68: 321-332.

Skov, F., Svenning, J.C. 2004. Potential impact of climatic change on the distribution of forest herbs in Europe. *Ecography*, 27: 366–380.

Sobhana, B., Nair, N.B. 1974. Observations on the maturation and spawning of *Puntius sarana* subnastus (Val.). *Indian Journal of Fisheries*, 21 (2): 357-369.

Somvanshi, 1983. Seasonal changes in biochemical composition of hill stream fish *Garra mullya*. *Indian Journal of Fisheries*, 30 (1).

Somvanshi, V.S. 1985. Indices of fecundity in the hill stream fish *Gurra mullay*. *Indian Journal of Fisheries*, 32(1): 33-43.

Spencer, H. 1864–1867. The principles of biology, 2 volumes. Williams & Norgate, 678 pp., London, U.K.

Sridhar, S., Vijaykumar, C., Haniffa, M.A. 1998. Induced spawning and establishment of a captive population for an endangered fish *Ompok bimaculatus* India. *Current Science*, 75: 1066-1068.

Srikar, L.N., Keshavanath, P., Peter, M. 1979. Changes in biochemical composition of *Clarius batarachus* in before and after spawning, *Mysore Journal of Agricultural Sciences*, 13: 84-96.

Srivastava, G.J. 1980. Fishes of U.P. and Bihar. Vishwavidyalaya Prakashan, Varanasi, India.

Stergiou, K.I., Moutopoulos, D.K. 2001. A review of length-weight relationships of fishes from Greek marine waters. Naga, ICLARM Quart, 24(1-2): 23-39.

Stoumbondi, M.T.W., Villwock, J.S., Abraham, M. 1993. Gonadosomatic index in *Borkus longiceps, Capoetado mascina* and their natural Hybrid (pisces: Cyprinidae) vercus spermatozoon index in the paternal males. *Journal of Fish Biology,* 43: 865-875.

Strauss, R.E., Bond, C.E. 1990. Taxonomic methods: Morphology. In: Schreck, C.B. and Moyle (Eds.) Methods for fish biology. American Fisheries society, Bethesda, Maryland., 109-140.

Sundararaj, B.I. Vasal, S. 1976. Photoperiod and temperature control in the regulation of reproduction in the female fish catfish, *Heteropneustes fossilis*. *Journal of the Fisheries Research Board of Canada,* 33. 959-973.

Suresh, V.R., Biswas, B.K., Vinci, G.K., Mukherjee, A. 2006. Biology and fishery of barred spiny eel, *Macrognathus pancalus* Hamilton. *Acta Ichthyologica Piscatoria,* 36: 31-37.

Surnar, S.R., Kamble, A.D., Walse, N.S., Sharma, O.P., Saini, V.P. 2015. Hormone administration with induced spawning of Indian major carp. *Internation journal of Fisheries and Aquatic Studies,* 3(1): 01-04.

Suwanjarat, J.T., Amornsakum, T., Thongboon, L., Boonyoung, P. 2005. Seasonal changes of spermatogenesis in the male sand goby *Oxyeleotris marmoratus* (Bleeker). *Journal of Science and Technology,* 27: 425-436.

Szczyglińska, A. 1980a. Analiza dwóch populacji okonia *Perca fluviatilis* (L.) i dwóch populacji płoci *Rutilus rutilus* (L.) pochodzących ze zbiorników o różnej termice wód. Zesz. Nauk ART Olsztyn, 10: 249–259.

Szczyglińska, A. 1980b. Cechy merystyczne płoci i okonia pochodzących ze zbiornika naturalne-go i termicznie zanieczyszczonego. Zesz. Nauk. ART Olsztyn, 10: 263–278.

Szollosi-Nagy. 2004. In Proceedings of the United Nations seminar, 25–26 November Delft, Netherlands.

Sztramko, L., Teleki, G.C. 1977. Annual variations in the fecundity of yellow perch from Long Point Bay, Lake Erie. *Transactions of the American Fishereis Society*, 106: 578-582.

Talwar, P.K., Jhingran A. 1991. Inland fishes of India and adjacent countries, 2 volumes. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, xix. 1158 pp.

Targonska, K., Kucharczyk, D., Zarski, D., Cejko, B., Krejszeff, S., Kupren, K., Krol, R., Dryl, K., Kowalski, R.K., Glogowski, J. 2011. Artificial reproduction of wild and cultured barbel (Barbus barbus, Cyprinidae) under controlled conditions. *Acta Veterinaria Hungarica,* 59(3): 363-72.

Taylor, E.B. 1986. Differences in morphology between wild and hatchery populations of juvenile coho salmon. *Progressive Fish-Culturist*, 48: 171–176.

Tesch, F.W. 1971. Age and growth. In: Ricker WE, ED. Methods for assessments of fish production in freshwaters. Blackwell Scientific Publication, Oxford, pp 98-130.

Thiry, M., Poncin, P. 2005. Morphological changes of the nucleolus during oogenesis in oviparous teleost fish, *Barbus barbus* (L.). *Journal of Structural Biology*, 152: 1-13.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, B., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Ferriera De Siqueira, M., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williams, S.E. 2004. Extinction risk from climate change. *Nature*, 427, 145–148.

Thomas, J., Venu, S., Kurup, B.M. 2003. Length-weight relationship of some deep-sea fish inhabiting the continental slope beyond 250m depth along the West coast of India, NAGA. *World Fish Center Quarterly*, 26(2): 17-21.

Thomas, K.R., George, M.J., Babu, C.R. 2002. Fresh water fishes of Southern Kerala with notes on the distribution of endemic and endangered species *Journal of the Bombay Natural History Society*, 99 (1): 47-53.

Thorpe, J.E., Talbot, C., Miles, M.S., Keay, D.S. 1990. Control of maturation in cultured Atlantic salmon, *Salmo salar* in pumped seawater tanks by restricting food intake. *Aquaculture*, 86: 315-326.

Tomkiewicz, J., Tybjerg, L., Jespersen, A. 2003. Micro and macroscopic characteristics to stage gonadal maturation of female *Baltic cod. Journal of Fish Biology*, 62: 253-275.

Treasurer, J.W. 1981. Some aspects of the reproductive biology of perch *Perca fluviatilis* L. fecundity, maturation and spawning behavior. *Fish Biology*, 18: 729-740.

Treasurer, J.W. 1990. The annual reproductive cycle of pike, *Esox Lucius* L. in two Scottish lakes *Journal of Fish Biology*, 36: 29-46.

Tsai, C., Gibson, G.R. 1971. Fecundity of the yellow perch, *Perca flavescens* Mitchill, in the Patuxent River, Maryland. *Chesapeake Science*, 12: 270-274.

Unal, G., Çetinkaya, O., Elp, M. 1999. Histological investigation of gonad development of *Chalcal burnustarichi. Turkish Journal of Zoology*, 23: 329-338.

Utoh, T., Horie, N., Okamura, A., Yamada, Y., Tanaka, S., Mikawa, N., Akazawa, A., Oka, H.P. 2003. Oogenesis in the common Japanese conger *Conger myriaster. Fisheries Science*, 69: 181–188.

Van Dyk, J.C., Pieterse, G.M. 2008. A histo-morphological study of the testis of the sharp tooth catfish (*Clarias gariepinus*) as reference for future toxicological assessments. *Journal of Applied Ichthyology*, 24: 415-422.

Vazzoler, A.E.A.M. 1996. Biologia da reproducao de peixesteleosteos: teoria e pratica. Maringa. EDIEM. 169p.

Verep, B., Turan, D., Kovac, V. 2006. Preliminary results on morphometry of babel (*Barbus tauricus*, Kessler, 1877) in the streams of Riza and Artvin province (Turkey). *Turkish Journal of Zoology*, 6: 17-21.

Verma, R. 2013. Seasonal changes in the histological profile of the testicular and ovarian cycle of *Labeo dyocheilus*. *International Journal of Fisheries andAquatic Studies*, 3: 143-149.

Vijayakumar, C. 2010. Conservation of an endangered Indian catfish *Ompok malabaricus* through captive breeding and establishment of captive population. *International Journal of Bio-Science and Bio-Technology*, 1(2): 131-133.

Villacorta-Correa, M.A., Saint-Paul, U. 1999. Structural indexes and sexual maturity of tambaqui *Colossoma macropomum* (Cuvier, 1818) (Characiformes: Characidae) in central Amazon, Brazil. *Revista Brasileira de Biologia*, 59: 637–652.

Virjenhock, A.I., Walford, L.A. and Vinci, G.K. 1998. Conservation genetics of fresh water fish. *Journal of Fish Biology*, 53: 394-412.

Von Cramon-Taubadel, S., Ling, E.N., Cotter, D., Wilkins, N.P. 2005. Determination of body-shape variation in Irish hatchery-reared and wild Atlantic salmon. *Journal of Fish Biology,* 66: 1471-1482

Vyas, V., Vishwakarma, K.S. 2013. Fish diversity in two tributaries of river Narmada, Central India. *Journal of Chemical, Biological and Physical Sciences,* 3(4): 2722-2730.

Walker, C.H. 1998. The use of biomarkers to measure the interactive effects of chemicals. *Ecotoxicology and Environmental Safety,* 40: 65–70.

Welcomme, R.L. 1985. River Fisheries. Food and Agriculture Organization, Technical Paper 262, Rome, Italy.

West, G. 1990. Methods of assessing ovarian development in fishes: A review. *Australian Journal of Marine and Freshwater Research.* 41:199-222.

WHO (World Health Organization). 1993. Guidelines for drinking water quality- I, recommendations, 2nd Ed. Geneva WHO. http:// www.lenntech.Com/drinking-water-standards.html.

Williams, S.E., Bolitho, E.E., Fox, S. 2003. Climate change in Australian tropical rainforests: an impending environmental catastrophe. Proc. R. Soc. Lond. B Biol. Sci., 270: 1887–1892.

Witthames, P.R., Walker, M.G. 1987. An automated method for counting and sizing fish eggs. *Journal of Fish Biology,* 30: 225-235.

Wooton, J.R. 1990. Ecology of teleost fishes. Chapman & Hall, London, England, 1990.

Wootton, R.S. 1992. Fish ecology. 1 st Ed., Thomson Litho Limited, Scotland, 203 pp.

Wright, R.M., Shoesmith, E.A. 1988. The reproductive success of pike, Esox lucius: Aspects of fecundity, egg density and survival. J. Fish Biol., 33: 623-636.

Yadav, N.S., Kumar, A., Sharma, M.P. 2014. Ecological health assessment of Chambal River using water quality parameters. *Journal of Integrated Science and Technology,* 2(2): 52-56.

Yasui, G.S., Senhorini, J.A., Shimoda, E., Pereira-Santos, M. 2015. Improvement of gamete quality and its short-term storage: an approach for biotechnology in laboratory fish. *Animal,* 9(3): 464-470.

Yelden, K., Avsar, S.R. 2000. Fecundities of some fresh water eel. *Journal of Zoology*, 10: 60-67.

Yuen, H.S.H. 1955. Maturity and fecundity of big-eye tuna in the Pacific. Spec. Sci. Rep. Fish., 150: 30.

Yusaf, M., Salam, A., Naeem, A. 2009. Length-weight and condition factor relationships of wild catfish, Wallago attu and Sperata sarrwari, from Indus River, Southern Punjab, Pakistan. *Journal of Applied Ichthyology,* 25: 614-61.

Zairin, M. Jr., Furukawa, Aida, K. 1992. Induction of ovulation by HCG injection in the tropical walking catfish *Clarius batrachus* under 23-25^{o}C. *Bulletin of the Japanese Society of Scientific Fisheries*, 59:1681-1695.

Zhan, Q.B., Wang, X.L. 2012. Elliptic Fourier analysis of the wing outline shape of five species of antlion (Neuroptera: Myrmeleontidae: Myrmeleontini). *Zoological Studies*, 51: 399-405.

Zonneveld, N., Viveen, W.J.A.R., Mudana, W. 1988. Induced spawning and egg incubation of the Asian catfish *Clarias batrachus. Aquaculture* 74: 41-47.

URKUND

Urkund Analysis Result

Analysed Document: anurag rawat.pdf (D30109513)
Submitted: 2017-08-14 16:21:00
Submitted By: shodhganga.bbau@gmail.com
Significance: 9 %

Sources included in the report:

http://www.fisheriesjournal.com/archives/%253Fyear%253D2016%2526vol%253D4%2526issue%253D3%2526part%253DF%2526ArticleId%253D779
http://docplayer.net/2860323-Arynopsis-f-iological-itata-on-the-perch-perca-fluviatilis-linnaeus-1758-and-perca-flavescens-mitchill-1814.html
https://www.researchgate.net/profile/Abha_Mishra10/publication/303684443_Spawning_dynamics_of_female_freshwater_butter_catfish_Ompok_bimaculatus_Bloch_1794_in_different_major_rivers_of_India/links/574d41a108ae82d2c6bc9127?origin=publication_list

Instances where selected sources appear:

71